A METHOD FOR THE PROPHYLAXIS AND/OR TREATMENT OF PROLIFERATIVE AND/OR INFLAMMATORY SKIN DISORDERS

The present invention relates generally to a method for the prophylaxis and/or treatment of skin disorders, and in particular proliferative and/or inflammatory skin disorders, and to genetic molecules useful for same. The present invention is particularly directed to genetic molecules capable of modulating growth factor interaction with its receptor on epidermal keratinocytes to inhibit, reduce or otherwise decrease stimulation of this layer of cells. The present invention contemplates, in a most preferred embodiment, a method for the prophylaxis and/or treatment of psoriasis.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Psoriasis and other similar conditions are common and often distressing proliferative and/or inflammatory skin disorders affecting or having the potential to affect a significant proportion of the population. The condition arises from over proliferation of basal keratinocytes in the epidermal layer of the skin associated with inflammation in the underlying dermis. Whilst a range of treatments have been developed, none is completely effective and free of adverse side effects. Although the underlying cause of psoriasis remains elusive, there is some consensus of opinion that the condition arises at least in part from over expression of local growth factors and their interaction with their receptors supporting keratinocyte proliferation *via* keratinocyte receptors which appear to be more abundant during psoriasis.

One important group of growth factors are the dermally-derived insulin-like growth factors (IGFs) which support keratinocyte proliferation. In particular, IGF-I and IGF-II are ubiquitous peptides each with potent mitogenic effects on a broad range of cells. Molecules of the IGF type are also known as "progression factors" promoting "competent" cells through DNA synthesis. The IGFs act through a common receptor known as the Type I or IGF-I receptor, which is tyrosine kinase linked. They are synthesised in mesenchymal tissues, including the dermis, and act on adjacent cells of mesodermal, endodermal or ectodermal origin. The regulation of their synthesis involves growth hormone (GH) in the liver, but is poorly defined in most tissues (1).

Particular proteins, referred to as IGF binding proteins (IGFBPs), appear to be involved in autocrine/paracrine regulation of tissue IGF availability (2). Six IGFBPs have so far been identified. The exact effects of the IGFBPs is not clear and observed effects *in vitro* have been inhibitory or stimulatory depending on the experimental method employed (3). There is some evidence, however, that certain IGFBPs are involved in targeting IGF-I to its cell surface receptor.

Skin, comprising epidermis and underlying dermis, has GH receptors on dermal fiboblasts (4). Fibroblasts synthesize IGF-I as well as IGFBPs-3, -4, -5 and -6 (5) which may be involved in targeting IGF-I to adjacent cells as well as to the overlaying epidermis. The major epidermal cell type, the keratinocyte, does not synthesize IGF-I, but possesses IGF-I receptors and is responsive to IGF-I (6).

It is apparent, therefore, that IGF-I and other growth promoting molecules, are responsible for or at least participate in a range of skin cell activities. In accordance with the present invention, the inventors have established that abberations in the normal functioning of these molecules or abberations in their interaction with their receptors is an important factor in proliferative and/or inflammatory skin disorders. It is proposed, therefore, to target these molecules or other molecules which facilitate their functioning or interaction with their receptors to thereby ameliorate the effects of abberant activity during or leading to skin disease conditions.

Accordingly, one aspect of the present invention contemplates a method for ameliorating the effects of a proliferative and/or inflammatory skin disorder in a mammal, said method comprising contacting the proliferating and/or inflamed skin or skin capable of proliferation and/or inflammation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing a growth factor mediated cell proliferation and/or inflammation.

Growth factor mediated cell proliferation and inflammation are also referred to as epidermal hyperplasias and may be mediated by any number of molecules such as but not limited to IGF-I, keratinocyte growth factor (KGF), transforming growth factor-α (TGFα), tumour necrosis factor-α (TNFα), interleukin-1, -4, -6 and 8 (IL-1, IL-4, IL-6 and IL-8, respectively), basic fibroblast growth factor (bFGF) or a combination of one or more of the above. The present invention is particularly described and exemplified with reference to IGF-I and its receptor (IGF-I receptor) and to IGF-I facilitating molecules, IGFBPs, since targeting these molecules according to the methods contemplated herein provides the best results to date. This is done, however, with the understanding that the present invention extends to any growth factor or cytokine-like molecule, a receptor thereof or a facilitating molecule like the IGFBPs involved in skin cell proliferation such as those molecules contemplated above and/or their receptors and/or facilitating molecules therefor.

According to this preferred embodiment, there is provided a method for ameliorating the effects of a proliferative and/or inflammatory skin disorder in a mammal, said method comprising contacting the proliferating and/or inflamed skin or skin capable of proliferation and/or inflammation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation and/or inflammation.

The present invention is particularly described by psoriasis as the proliferative skin disorder. However, the subject invention extends to a range of proliferative and/or inflammatory skin disorders or epidermal hyperplasias such as but not limited to psoriasis, ichthyosis, pityriasis rubra pilaris ("PRP"), seborrhoea, keloids, keratoses,

neoplasias and scleroderma, warts, benign growths and cancers of the skin.

In a preferred embodiment, therefore, the present invention is directed to a method for ameliorating the effects of psoriasis, said method comprising contacting proliferating skin or skin capable of proliferation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation.

The present invention extends to any mammal such as but not limited to humans, livestock animals (e.g. horses, sheep, cows, goats, pigs, donkeys), laboratory test animals (e.g. rabbits, mice, guinea pigs), companion animals (e.g. cats, dogs) and captive wild animals. However, the instant invention is particularly directed to proliferative and/or inflammatory skin disorders such as psoriasis in humans.

The aspects of the subject invention instantly contemplated are particularly directed to the topical application of one or more suitable nucleic molecules capable of inhibiting, reducing or otherwise interfering with IGF-mediated cell proliferation and/or inflammation. More particularly, the nucleic acid molecule targets IGF-I interaction with its receptor. Conveniently, therefore, the nucleic acid molecule is an antagonist of IGF-I interaction with its receptor. Most conveniently, the nucleic acid molecule antagonist is an antisense molecule to the IGF-I receptor, to IGF-I itself or to a molecule capable of facilitating IGF-I interaction with its receptor such as but not limited to an IGFBP.

Insofar as the invention relates to IGFBPs, the preferred molecules are IGFBP-2, -3, -4, -5 and -6. The most preferred molecules are IGFBP-2 and IGFBP-3.

The nucleotide sequences of IGFBP-2 and IGFBP-3 are set forth in Figures 1 (SEQ ID NO. 1) and 2 (SEQ ID NO. 2), respectively. According to a particularly preferred aspect of the present invention, there is provided a nucleic acid molecule comprising at least about ten nucleotides capable of hybridising to, forming a heterodouplex or otherwise interacting with an mRNA molecule directed from a gene corresponding to

a genomic form of SEQ ID NO. 1 and/or SEQ ID NO. 2 and which thereby reduces or inhibits translation of said mRNA molecule. Preferably, the nucleic acid molecule is at least about 15 nucleotides in length and more preferably at least about 20-25 nucleotides in length. However, the instant invention extends to any length nucleic acid molecule including a molecule of 100-200 nucleotides in length to correspond to the full length of or near full length of the subject genes.

The nucleotide sequence of the antisense molecules may correspond exactly to a region or portion of SEQ ID NO. 1 or SEQ ID NO. 2 or may differ by one or more nucleotide substitutions, deletions and/or additions. It is a requirement, however, that the nucleic acid molecule interact with an mRNA molecule to thereby reduce its translation into active protein.

Examples of potential antisense molecules for IGFBP-2 and IGFBP-3 are those capable of interacting with sequences selected from the lists in Examples 6 and 7, respectively.

The nucleic acid molecules in the form of an antisense molecule may be linear or covalently closed circular and single stranded or partially double stranded. A double stranded molecule may form a triplex with target mRNA or a target gene. The molecule may also be protected from, for example, nucleases, by any number of means such as using a nonionic backbone or a phosphorothioate linkage. A convenient nonionic backbone contemplated herein is ethylphosphotriester linkage or a 2'-O-methylribosyl derivative.

Examples of suitable oligonucleotide analogues are conveniently described in Ts'O *et al* (7).

Alternatively, the antisense molecules of the present invention may target the IGF-I gene itself or its receptor or a multivalent antisense molecule may be constructed or separate molecules administered which target at least two or an IGFBP, IGF-I and/or IGF-I-receptor. Examples of suitable antisense molecules capable of targetting the IGF-I receptor are those capable of interacting with sequences selected from the list in

Example 8. One particularly useful antisense molecule is 5'- ATCTCTCCGCTTCCTTTC -3' (SEQ ID NO. 10). A particularly preferred embodiment of the present invention contemplates a method of ameliorating the effects of psoriasis, said method comprising contacting proliferating skin or skin capable of proliferation with an effective amount of one or more nucleic acid molecules or chemical analogues thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation wherein said one or more molecules comprises a polynucleotide capable of interacting with mRNA directed from two or more of an IGF-I gene, an IGF-I receptor gene or a gene encoding an IGFBP such as IGFBP-2 and/or IGFBP-3.

In accordance with one aspect of the present invention the nucleic acid molecule is topically applied in aqueous solution or in conjunction with a cream, ointment, oil or other suitable carrier and/or diluent. A single application may be sufficient depending on the severity or exigencies of the condition although more commonly, multiple applications are required ranging from hourly, multi-hourly, daily, multi-daily, weekly or monthly, or in some other suitable time interval. The treatment might comprise solely the application of the nucleic acid molecule or this may be applied in conjunction with other treatments for the skin proliferation and/or inflammatory disorder being treated or for other associated conditions including microbial infection, bleeding and the formation of a variety of rashes.

As an alternative to or in conjunction with antisense therapy, the subject invention extends to the nucleic acid molecule as, or incorporating, a ribozyme including a minizyme to, for example, IGF-I, its receptor or to molecules such as IGFBPs and in particular IGFBP-2 and -3. Ribozymes are synthetic nucleic acid molecules which possess highly specific endoribonuclease activity. In particular, they comprise a hybridising region which is complementary in nucleotide sequence to at least part of a target RNA. Ribozymes are well described by Haseloff and Gerlach (8) and in International Patent Application No. WO 89/05852. The present invention extends to ribozymes which target mRNA specified by genes encoding IGF-I, its receptor or one or more IGFBPs such as IGFBP-2 and/or IGFBP-3.

According to this embodiment, there is provided in a particularly preferred aspect a ribozyme comprising a hybridising region and a catalytic region wherein the hybridising region is capable of hybridising to at least part of a target mRNA sequence transcribed from a genomic gene corresponding to SEQ ID NO. 1 or SEQ ID NO. 2 wherein said catalytic domain is capable of cleaving said target mRNA sequence to reduce or inhibit IGF-I mediated cell proliferation and/or inflammation.

Yet another aspect of the present invention contemplates co-suppression to reduce expression or to inhibit translation of an endogenous gene encoding, for example, IGF-I, its receptor, or IGFBPs such as IGFBP-2 and/or -3. In co-suppression, a second copy of an endogenous gene or a substantially similar copy or analogue of an endogenous gene is introduced into a cell following topical administration. As with antisense molecules, nucleic acid molecules defining a ribozyme or nucleic acid molecules useful in co-suppression may first be protected such as by using a nonionic backbone.

The efficacy of the nucleic acid molecules of the present invention can be conveniently tested and screened using an *in vitro* system comprising a basal keratinocyte cell line. A particularly useful system comprises the HaCaT cell line described by Boukamp *et al* (9). In one assay, IGF-I is added to an oligonucleotide treated HaCaT cell line. Alternatively, growth of oligonucleotide treated HaCaT cells is observed on a feeder layer of irradiated 3T3 fibroblasts. Using such *in vitro* assays, it is observed that antisense oligonucleotides to IGFBP-3, for example, inhibit production of IGFBP-3 by HaCaT cells. Other suitable animal models include the nude mouse/human skin graft model (15; 16) and the "flaky skin" mouse model (17; 18). In the nude mouse model, microdermatome biopsies of psoriasis lesions are taken under local anaesthetic from volunteers then transplanted to congenital athymic (nude) mice. These transplanted human skin grafts maintain the characteristic hyperproliferating epidermis for 6-8 weeks. They are an established model for testing the efficacy of topically applied therapies for psoriasis. In the "flaky skin" mouse model, the fsn/fsn mutation produces mice with skin resembling human psoriasis. This mouse, or another mutant mouse with a similar phenotype is a further *in vivo* model to test the efficacy of topically applied therapies for psoriasis.

Another aspect of the present invention contemplates a pharmaceutical composition for topical administration which comprises a nucleic acid molecule capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation such as psoriasis and one or more pharmaceutically acceptable carriers and/or diluents. Preferably, the nucleic acid molecule is an antisense molecule to IGF-I, the IGF-I receptor or an IGFBP such as IGFBP-2 and/or IGFBP-3 or comprises a ribozyme to one or more of these targets or is a molecule suitable for co-suppression of one or more of these targets. The composition may comprise a single species of a nuleic acid molecule capable of targeting one of IGF-I, its receptor or an IGFBP, such as IGFBP-2 or IGFBP-3 or may be a multi-valent molecule capable of targeting two or more of IGF-I, its receptor or an IGFBP, such as IGFBP-2 and/or IGFBP-3.

The nucleic acid molecules may be administered in dispersions prepared in creams, ointments, oil or other suitable carrier and/or diluent such as glycerol, liquid polyethylene glycols and/or mixtures thereof. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for topical use include sterile aqueous solutions (where water soluble) or dispersions and powders for the extemporaneous preparation of topical solutions or dispersion. In all cases, the form is preferably sterile although this is not an absolute requirement and is stable under the conditions of manufacture and storage. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The prevention of the action of microorganism can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

Topical solutions are prepared by incorporating the nucleic acid molecule compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by where necessary filter sterilization.

As used herein "pharmaceutically acceptable carriers and/or diluents" include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatable with the active ingredient, use thereof in the pharmaceutical compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. Conveniently, the nucleic acid molecules of the present invention are stored in freeze-dried form and are reconstituted prior to use.

Yet another aspect of the present invention contemplates the use of a nucleic acid molecule in the manufacture of a medicament for the treatment of proliferative and/or inflammatory skin disorders mediated by a growth factor. The proliferative and/or inflammatory skin disorder is generally psoriasis and the nucleic acid molecule targets IGF-I, the IGF-I receptor and/or an IGFBP such as IGFBP-2 and/or IGFBP-3.

Still a further aspect of the present invention contemplates an agent comprising a nucleic acid molecule as hereinbefore defined useful in the treatment of proliferative and/or inflammatory skin disorders, such as psoriasis.

The present invention is further described by the following non-limiting Figures and/or Examples.

In the Figures:

Figure 1 is a representation of the nucleotide sequence of IGFBP-2.

```
LOCUS       HSIGFBP2     1433 bp    RNA              PRI        31-JAN-1990
DEFINITION  Human mRNA for insulin-like growth factor binding protein (IGFBP-2)
ACCESSION   X16302
KEYWORDS    insulin-like growth factor binding protein.
SOURCE      human
  ORGANISM  Homo sapiens
            Eukaryota; Animalia; Metazoa; Chordata; Vertebrata; Mammalia;
            Theria; Eutheria; Primates; Haplorhini; Catarrhini; Hominidae.
REFERENCE   1  (bases 1 to 1433)
  AUTHORS   Binkert,C., Landwehr,J., Mary,J.L., Schwander,J. and Heinrich,G.
  TITLE     Cloning, sequence analysis and expression of a cDNA encoding a
            novel insulin-like growth factor binding protein (IGFBP-2)
  JOURNAL   EMBO J. 8, 2497-2502 (1989)
  STANDARD  full automatic
COMMENT     NCBI gi: 33009
FEATURES             Location/Qualifiers
     source          1. .1433
                     /organism="Homo sapiens"
                     /dev_stage="fetal"
                     /tissue_type="liver"
     misc_feature    1416. .1420
                     /note="pot. polyadenylation signal"
     polyA_site      1433
                     /note="polyadenylation site"
     CDS             118. .1104
                     /note="precursor polypeptide;  (AA -39 to 289);  NCBI gi:
                     33010."
                     /codon_start=1
                     /translation="MLPRVGCPALPLPPPPLLPLLPLLLLLLGASGGGGGARAEVLFR
                     CPPCTPERLAACGPPPVAPPAAVAAVAGGARMPCAELVREPGCGCCSVCARLEGEACG
                     VYTPRCGQGLRCYPHPGSELPLQALVMGEGTCEKRRDAEYGASPEQVADNGDDHSEGG
                     LVENHVDSTMNMLGGGGSAGRKPLKSGMKELAVFREKVTEQHRQMGKGGKHHLGLEEP
                     KKLRPPPARTPCQQELDQVLERISTMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCK
                     MSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQQEACGVHTQRMQ"
     CDS             118. .234
                     /note="signal peptide;  (AA -39 to -1);  NCBI gi: 33011."
                     /codon_start=1
                     /translation="MLPRVGCPALPLPPPPLLPLLPLLLLLLGASGGGGGARA"
     CDS             235. .1101
                     /note="mature IGFBP-2;  (AA 1 to 289);  NCBI gi: 33012."
                     /codon_start=1
                     /translation="EVLFRCPPCTPERLAACGPPPVAPPAAVAAVAGGARMPCAELVR
                     EPGCGCCSVCARLEGEACGVYTPRCGQGLRCYPHPGSELPLQALVMGEGTCEKRRDAE
                     YGASPEQVADNGDDHSEGGLVENHVDSTMNMLGGGGSAGRKPLKSGMKELAVFREKVT
                     EQHRQMGKGGKHHLGLEEPKKLRPPPARTPCQQELDQVLERISTMRLPDERGPLEHLY
                     SLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNE
                     QQEACGVHTQRMQ"
BASE COUNT      239 a    466 c    501 g    227 t
ORIGIN

 HSIGFBP2  Length: 1433  May 11, 1994  10:06  Type: N  Check: 6232  ..
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Figure 2 is a representation of the nucleotide sequence of IGFBP-3.

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LOCUS       HUMGFIBPA     2474 bp ss-mRNA            PRI       15-JUN-1990
DEFINITION  Human growth hormone-dependent insulin-like growth factor-binding
            protein mRNA, complete cds.
ACCESSION   M31159
KEYWORDS    insulin-like growth factor binding protein.
SOURCE      Human plasma, cDNA to mRNA, clone BP-53.
  ORGANISM  Homo sapiens
            Eukaryota; Animalia; Chordata; Vertebrata; Mammalia; Theria;
            Eutheria; Primates; Haplorhini; Catarrhini; Hominidae.
REFERENCE   1  (bases 1 to 2474)
  AUTHORS   Wood,W.I., Cachianes,G., Henzel,W.J., Winslow,G.A., Spencer,S.A.,
            Hellmiss,R., Martin,J.L. and Baxter,R.C.
  TITLE     Cloning and expression of the growth hormone-dependent insulin-like
            growth factor-binding protein
  JOURNAL   Mol. Endocrinol. 2, 1176-1185 (1988)
  STANDARD  full automatic
COMMENT     NCBI gi: 183115
FEATURES             Location/Qualifiers
     mRNA            <1. .2474
                     /note="GFIBP mRNA"
     CDS             110. .985
                     /gene="IGFBP1"
                     /note="insulin-like growth factor-binding protein;  NCBI
                     gi: 183116."
                     /codon_start=1
                     /translation="MQRARPTLWAAALTLLVLLRGPPVARAGASSGGLGPVVRCEPCD
                     ARALAQCAPPPAVCAELVREPGCGCCLTCALSEGQPCGIYTERCGSGLRCQPSPDEAR
                     PLQALLDGRGLCVNASAVSRLRAYLLPAPPAPGNASESEEDRSAGSVESPSVSSTHRV
                     SDPKFHPLHSKIIIIKKGHAKDSQRYKVDYESQSTDTQNFSSESKRETEYGPCRREME
                     DTLNHLKFLNVLSPRGVHIPNCDKKGFYKKKQCRPSKGRKRGFCWCVDKYGQPLPGYT
                     TKGKEDVHCYSMQSK"
     source          1. .2474
                     /organism="Homo sapiens"
BASE COUNT      597 a     646 c     651 g     580 t
ORIGIN

 HUMGFIBPA  Length: 2474  May 11, 1994  10:00  Type: N  Check: 9946  ..
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Figure 3 is a representation of the nucleotide sequence of IGF-1-receptor.

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LOCUS       HSIGFIRR      4989 bp    RNA             PRI       28-MAR-1991
DEFINITION  Human mRNA for insulin-like growth factor I receptor
ACCESSION   X04434 M24599
KEYWORDS    glycoprotein; insulin receptor;
            insulin-like growth factor I receptor; membrane glycoprotein;
            receptor; tyrosine kinase.
SOURCE      human
  ORGANISM  Homo sapiens
            Eukaryota; Animalia; Metazoa; Chordata; Vertebrata; Mammalia;
            Theria; Eutheria; Primates; Haplorhini; Catarrhini; Hominidae.
REFERENCE   1  (bases 1 to 4989)
  AUTHORS   Ullrich,A., Gray,A., Tam,A.W., Yang-Feng,T., Tsubokawa,M.,
            Collins,C., Henzel,W., Bon,T.L., Kathuria,S., Chen,E., Jakobs,S.,
            Francke,U., Ramachandran,J. and Fujita-Yamaguchi,Y.
  TITLE     Insulin-like growth factor I receptor primary structure: comparison
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               with insulin receptor suggests structural dererminants that define
               functional specificity
  JOURNAL      EMBO J. 5, 2503-2512 (1986)
  STANDARD     full automatic
COMMENT        NCBI gi: 33058
FEATURES                Location/Qualifiers
     source             1. .4989
                        /organism="Homo sapiens"
                        /tissue_type="placenta"
                        /clone_lib="(lamda)gt10"
                        /clone="(lambda)IGF-1-R.85, (lambda)IGF-1-R.76"
     sig_peptide        32. .121
     mat_peptide        122. .4132
                        /note="IGF-I receptor"
     misc_feature       122. .2251
                        /note="alpha-subunit (AA 1 - 710)"
     misc_feature       182. .190
                        /note="pot.N-linked glycosylation site (AA 21 - 23)"
     misc_feature       335. .343
                        /note="pot.N-linked glycostlation site (AA 72 - 74)"
     misc_feature       434. .442
                        /note="pot.N-linked glycostlation site (AA 105 - 107)"
     misc_feature       761. .769
                        /note="pot.N-linked glycostlation site (AA 214 - 216)"
     misc_feature       971. .979
                        /note="pot.N-linked glycostlation site (AA 284 - 286)"
     misc_feature       1280. .1288
                        /note="pot.N-linked glycostlation site (AA 387 - 389)"
     misc_feature       1343. .1351
                        /note="pot.N-linked glycosylation site (AA 408 - 410)"
     misc_feature       1631. .1639
                        /note="pot.N-linked glycostlation site (AA 504 - 506)"
     misc_feature       1850. .1858
                        /note="pot.N-linked glycosylation site (AA 577 - 579)"
     misc_feature       1895. .1903
                        /note="pot.N-linked glycosylation site (AA 592 - 594)"
     misc_feature       1949. .1957
                        /note="pot.N-linked glycosylation site (AA 610 - 612)"
     misc_feature       2240. .2251
                        /note="putative proreceptor processing site (AA 707 -
                        710)"
     misc_feature       2252. .4132
                        /note="beta-subunit (AA 711 - 1337)"
     misc_feature       2270. .2278
                        /note="pot.N-linked glycosylation site (AA 717 - 719)"
     misc_feature       2297. .2305
                        /note="pot.N-linked glycosylation site (AA 726 - 728)"
     misc_feature       2321. .2329
                        /note="pot.N-linked glycosylation site (AA 734 - 736)"
     misc_feature       2729. .2737
                        /note="pot.N-linked glycosylation site (AA 870 - 872)"
     misc_feature       2768. .2776
                        /note="pot.N-linked glycosylation site (AA 883 - 885)"
     misc_feature       2837. .2908
                        /note="transmembrane region (AA 906 - 929)"
     misc_feature       2918. .2926
                        /note="pot.N-linked glycosylation site (AA 933 - 935)"
     misc_feature       3047. .3049
                        /note="pot.ATP binding site (AA 976)"
     misc_feature       3053. .3055
                        /note="pot.ATP binding site (AA 978)"
     misc_feature       3062. .3064
                        /note="pot.ATP binding site (AA 981)"
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        misc_feature     3128. .3130
                         /note="pot.ATP binding site (AA 1003)"
        CDS              32. .4132
                         /product="IGF-I receptor"
                         /note="50 stops when translation attempted, frame 1, code
                         0"
   BASE COUNT      1216 a    1371 c    1320 g    1082 t
   ORIGIN

     HSIGFIRR  Length: 4989  May 11, 1994  12:10  Type: N  Check: 133  ..
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Figure 4A is a photographic representation of a Western ligand blot of HaCaT conditioned medium showing IGFBP-3 secreted in 24 hours after 7 day treatment with phosphorothioate oligonucleotides (BP3AS2, BP3AS3 and BP3S) at 0.5μM and 5μM; * no oligonucleotide added.

Figure 4B is a graphical representation of a scanning imaging desitometry of Western ligand blot (Figure 4A), showing relative band intensities of IGFBP-3 and the 24kDa IGFBP-4 after treatment with phosphorothioate oligonucleotides; * no oligonucleotide added.

Figure 5A is a photographic representation of a Western ligand blot of HaCaT conditioned medium showing IGFBP-3 secreted in 24 hours after 7 day treatment with phosophorothioate oligonucleotide BP3AS2 at 0.5μM compared with several control oligonucleotides at 0.5μM. (a) oligonucleotide BP3AS2NS; (b) oligonucleotide BP3AS4; (c) oligonucleotide BP3AS4NS; and (untreated), no oligonucleotide added.

Figure 5B is a graphical representation of a scanning imaging densitometry of Western ligand blot (Figure 5A), showing relative band intensities of IGFBP-3 after treatment with phosphorothioate oligonucleotides as in Figure 5A, showing IGFBP-3 band intensities expressed as a percentage of the average band intensity from conditioned medium of cells not treated with oligonucleotide.

Figure 6 is a graphical representation showing inhibition of IGF-I binding by antisense oligonucleotides to IGF-I receptor. IGFR.AS: antisense; IGFR.S: sense.

Figure 7 is a graphical representation showing inhibition of IGFBP-3 production in culture medium following initial treatment with antisense oligonucleotides once daily over a 2 day period.

Figure 8 is a graphical representation showing optimization of IGFBP-3 antisense oligonucleotide concentration as determined by relative IGFBP-3 concentration in culture medium.

EXAMPLE 1

IN VITRO ASSAY: CELLS

The differentiated human keratinocyte cell line, HaCaT (9) was used in the *in vitro* assay. Cells at passage numbers 33 to 36 were maintained as monolayer cultures in 5% v/v CO_2 at 37°C in Keratinocyte-SFM (Gibco) containing EGF and bovine pituitary extract as supplied. Media containing foetal calf serum were avoided because of the high content of IGF-I binding proteins in serum.

Feeder layer plates of lethally irradiated 3T3 fibroblasts were prepared exactly as described by Rheinwald and Green (10).

EXAMPLE 2

IN VITRO ASSAY: THYMIDINE INCORPORATION ASSAY

Cells were grown to 4 days post confluence in $2cm^2$ wells with daily medium changes of Keratinocyte-SFM, then the medium was changed to DMEM (Cytosystems, Australia), with the following additions: 25mM Hepes, 0.19% w/v, sodium bicarbonate, 0.03% w/v glutamine (Sigma Chemical Co, USA), 50IU/ml penicillin and 50μg/ml streptomycin (Flow Laboratories). After 24 hours, IGF-I or tIGF-I was added to triplicate wells, at the concentrations indicated, in 0.5ml fresh DMEM containing 0.02% v/v bovine serum albumin (Sigma molecular biology grade) and incubated for a further 21 hours. $[^3H]$-Thymidine (0.1μCi/well) was then added and the cells incubated for a further 3 hours. The medium was then aspirated and the cells washed once with ice-cold PBS and twice with ice-cold 10% v/v TCA. The TCA-precipitated monolayers were

then solubilized with 0.25M NaOH (200µl/well), transferred to scintillation vials and radioactivity determined by liquid scintillation counting (Pharmacia Wallac 1410 liquid scintillation counter).

EXAMPLE 3

WESTERN LIGAND BLOTTING

HaCaT conditioned medium (250µl) was concentrated by adding 750µl cold ethanol, incubating at -20°C for 2 hours and centrifuging at 16,000g for 20 min at 4°C. The resulting pellet was air dried, resuspended thoroughly in non-reducing Laemmli sample buffer, heated to 90°C for 5 minutes and separated on 12% w/v SDS-PAGE according to the method of Laemmli (1970). Separated proteins were electrophoretically transferred to nitrocellulose membrane (0.45mm, Schleicher and Schuell, Dassel, Germany) in a buffer containing 25mM Tris, 192mM glycine and 20% v/v methanol. IGFBPs were then visualised by the procedure of Hossenlopp *et al* (11), using [^{125}I]-IGF-I, followed by autoradiography. Autoradiographs were scanned in a BioRad Model GS-670 Imaging Densitometer and band densities were determined using the Molecular Analyst program.

EXAMPLE 4

ANTISENSE OLIGONUCLEOTIDES

Phosphorothioate oligodeoxynucleotides were synthesised by Bresatec, Adelaide, South Australia, Australia. The following antisense sequences were used: BP3AS2, 5′- GCG CCC GCT GCA TGA CGC CTG CAA C -3′ (SEQ ID NO. 4), a 25mer complementary to the start codon region of the human IGFBP-3 mRNA; BP3AS3, 5′- CGG GCG GCT CAC CTG GAG CTG GCG -3′ (SEQ ID NO. 5), a 24mer complementary to the exon 1/intron 1 splice site; BP3AS4, 5′- AGG CGG CTG ACG GCA CTA -3′ (SEQ ID NO. 6), an 18mer complementary to a region of the coding sequence lacking RNA secondary structure and oligonucleotide-dimer formation (using the computer software "OLIGO for PC"). Since BP3AS4 was found to be ineffective at inhibiting IGFBP-3 synthesis, it was used as a control. The following additional control oligonucleotide sequences were used: BP3S, 5′- CAG GCG TCA TGC AGC GGG C -3′ (SEQ ID NO. 7), an 18mer sense control sequence equivalent to the start codon region; BP3AS2NS, 5′- CGG AGA

TGC CGC ATG CCA GCG CAG G -3′ (SEQ ID NO. 8), a 25mer randomised sequence with the same GC content as BP3AS2; BP3AS4NS, 5′- GAC AGC GTC GGA GCG ATC -3′ (SEQ ID NO. 9), an 18mer randomised sequence with the same GC content as BP3AS4NS. Design of the oligonucleotides was based on the human IGFBP-3 cDNA sequence of Spratt *et al* (12).

Cells were grown to one day post confluence in $2cm^2$ wells with daily medium changes of 0.5ml Keratinocyte-SFM, then subjected to daily medium changes of Keratinocyte-SFM for a further 4 days. Daily additions of 0.5ml fresh Keratinocyte-SFM were then continued for a further 7 days, except that at the time of medium addition, 5μl oligonucleotide in PBS was added to give the final concentrations indicated, then the wells were shaken to mix the oligonucleotide. After the final addition, cells were incubated for 24 hours and the medium collected for assay of IGFBPs. Cells were then counted after trypsinisation in a Coulter Industrial D Counter, Coulter Bedfordshire, UK. Cell numbers after oligonucleotide treatment differed by less than 10%.

EXAMPLE 5

ANTISENSE OLIGONUCLEOTIDES INHIBIT IGFBP-3 SYNTHESIS

HaCaT cells secrete mainly IGFBP-3 (>95%), with the only other IGFBP detectable in HaCaT conditioned medium being IGFBP-4 (<5%). The effect on IGFBP-3 and IGFBP-4 synthesis of antisense oligonucleotides at two concentrations, 5μM and 0.5μM, was tested. Two oligonucleotides were used, BP3AS2 and BP3AS3, directed against the start site and the intron 1/exon 1 splice site, respectively of the IGFBP-3 mRNA. As a control, a sense oligonucleotide corresponding to the start site was used. As shown in Figures 4A and 4B, all oligonucleotides at 5μM caused a significant reduction of IGFBP-3 synthesis compared with untreated cells, however, the two antisense oligonucleotides inhibited IGFBP-3 synthesis of approximately 50% compared to the sense control (Figure 4B). The antisense oligonucleotide directed to the start codon appeared to be more effective of the two, the difference being more apparent at the lower concentration of 0.5μM. The cells of IGFBP-4 secreted by the HaCaT cells make photographic reproduction of the bands on Western ligand blots difficult, however densitometry measurements provide adequate relative quantitation. This resulted in the

significant observation that IGFBP-4 levels were unaffected by oligonucleotide addition to the cells, suggesting that the observed inhibitory effects on IGFBP-3 are specific.

To further investigate the inhibitory effects of the more effective of the two antisense oligonucleotides, BP3AS2, inhibition by this oligonucleotide at 0.5μM was compared with a number of control oligonucleotides, including one antisense oligonucleotide to IGFBP-3 that had proved to be ineffective at 0.5μM. As shown in Figures 5A and 5B, BP3AS2 was again inhibitory, resulting in levels of IGFBP-3 of approximately 50% of the most non-specifically inhibitory control oligonucleotide, the randomised equivalent of BP3AS2. The other control oligonucleotides caused no reduction in IGFBP-3 levels at 0.5μM, compared to untreated cells. Of possible significance is the fact that this control oligonucleotide, BP3AS2NS, like BP3AS2 itself, has the highest potential T_m of the three control oligonucleotides used in this experiment, enhancing the probability of non-specific base pairing with non-target mRNAs. However, the lack of inhibition of IGFBP-4 secretion by BP3AS2 suggests that this oligonucleotide is selective even compared with the most closely related protein likely to be present in this cell line.

EXAMPLE 6

ANTISENSE OLIGONUCLEOTIDES OF IGFBP2

Antisense oligonucleotides to IGFBP2 may be selected from molecules capable of interacting with one or more of the following sense oligonucleotides:

ATTCGGGGCGAGGGA	AAGAAGCGGAGGAGG	CCCGCTCGCAGGGCC
TTCGGGGCGAGGGAG	AGAAGCGGAGGAGGC	CCGCTCGCAGGGCCG
TCGGGGCGAGGGAGG	GAAGCGGAGGAGGCG	CGCTCGCAGGGCCGT
CGGGGCGAGGGAGGA	AAGCGGAGGAGGCGG	GCTCGCAGGGCCGTG
GGGGCGAGGGAGGAG	AGCGGAGGAGGCGGC	CTCGCAGGGCCGTGC
GGGCGAGGGAGGAGG	GCGGAGGAGGCGGCT	TCGCAGGGCCGTGCA
GGCGAGGGAGGAGGA	CGGAGGAGGCGGCTC	CGCAGGGCCGTGCAC
GCGAGGGAGGAGGAA	GGAGGAGGCGGCTCC	GCAGGGCCGTGCACC
CGAGGGAGGAGGAAG	GAGGAGGCGGCTCCC	CAGGGCCGTGCACCT
GAGGGAGGAGGAAGA	AGGAGGCGGCTCCCG	AGGGCCGTGCACCTG
AGGGAGGAGGAAGAA	GGAGGCGGCTCCCGC	GGGCCGTGCACCTGC
GGGAGGAGGAAGAAG	GAGGCGGCTCCCGCT	GGCCGTGCACCTGCC
GGAGGAGGAAGAAGC	AGGCGGCTCCCGCTC	GCCGTGCACCTGCCC
GAGGAGGAAGAAGCG	GGCGGCTCCCGCTCG	CCGTGCACCTGCCCG
AGGAGGAAGAAGCGG	GCGGCTCCCGCTCGC	CGTGCACCTGCCCGC
GGAGGAAGAAGCGGA	CGGCTCCCGCTCGCA	GTGCACCTGCCCGCC
GAGGAAGAAGCGGAG	GGCTCCCGCTCGCAG	TGCACCTGCCCGCCC
AGGAAGAAGCGGAGG	GCTCCCGCTCGCAGG	GCACCTGCCCGCCCG
GGAAGAAGCGGAGGA	CTCCCGCTCGCAGGG	CACCTGCCCGCCCGC
GAAGAAGCGGAGGAG	TCCCGCTCGCAGGGC	ACCTGCCCGCCCGCC

CCTGCCCGCCCGCCC
CTGCCCGCCCGCCCG
TGCCCGCCCGCCCGC
GCCCGCCCGCCCGCT
CCCGCCCGCCCGCTC
CCGCCCGCCCGCTCG
CGCCCGCCCGCTCGC
GCCCGCCCGCTCGCT
CCCGCCCGCTCGCTC
CCGCCCGCTCGCTCG
CGCCCGCTCGCTCGC
GCCCGCTCGCTCGCT
CCCGCTCGCTCGCTC
CCGCTCGCTCGCTCG
CGCTCGCTCGCTCGC
GCTCGCTCGCTCGCC
CTCGCTCGCTCGCCC
TCGCTCGCTCGCCCG
CGCTCGCTCGCCCGC
GCTCGCTCGCCCGCC
CTCGCTCGCCCGCCG
TCGCTCGCCCGCCGC
CGCTCGCCCGCCGCG
GCTCGCCCGCCGCGC
CTCGCCCGCCGCGCC
TCGCCCGCCGCGCCG
CGCCCGCCGCGCCGC
GCCCGCCGCGCCGCG
CCCGCCGCGCCGCGC
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CGCCGCGCCGCGCTG
GCCGCGCCGCGCTGC
CCGCGCCGCGCTGCC
CGCGCCGCGCTGCCG
GCGCCGCGCTGCCGA
CGCCGCGCTGCCGAC
GCCGCGCTGCCGACC
CCGCGCTGCCGACCG
CGCGCTGCCGACCGC
GCGCTGCCGACCGCC
CGCTGCCGACCGCCA
GCTGCCGACCGCCAG
CTGCCGACCGCCAGC
TGCCGACCGCCAGCA
GCCGACCGCCAGCAT
CCGACCGCCAGCATG
CGACCGCCAGCATGC
GACCGCCAGCATGCT
ACCGCCAGCATGCTG
CCGCCAGCATGCTGC
CGCCAGCATGCTGCC
GCCAGCATGCTGCCG
CCAGCATGCTGCCGA
CAGCATGCTGCCGAG
AGCATGCTGCCGAGA
GCATGCTGCCGAGAG
CATGCTGCCGAGAGT
ATGCTGCCGAGAGTG
TGCTGCCGAGAGTGG
GCTGCCGAGAGTGGG
CTGCCGAGAGTGGGC
TGCCGAGAGTGGGCT
GCCGAGAGTGGGCTG
CCGAGAGTGGGCTGC
CGAGAGTGGGCTGCC
GAGAGTGGGCTGCCC
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GCCCCGCGCTGCCGC
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CCCGCGCTGCCGCTG
CCGCGCTGCCGCTGC
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GCGCTGCCGCTGCCG
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CTGCTACTGGGCGCG
TGCTACTGGGCGCGA
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GGGCGCGCGCGGAGG
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CGCGCGGAGGTGCTG
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CGGAGGTGCTGTTCC
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GAGGTGCTGTTCCGC
AGGTGCTGTTCCGCT
GGTGCTGTTCCGCTG
GTGCTGTTCCGCTGC
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GCCCGCCCTGCACAC
CCCGCCCTGCACACC
CCGCCCTGCACACCC
CGCCCTGCACACCCG
GCCCTGCACACCCGA
CCCTGCACACCCGAG
CCTGCACACCCGAGC
CTGCACACCCGAGCG
TGCACACCCGAGCGC
GCACACCCGAGCGCC
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ACACCCGAGCGCCTG
CACCCGAGCGCCTGG
ACCCGAGCGCCTGGC
CCCGAGCGCCTGGCC
CCGAGCGCCTGGCCG
CGAGCGCCTGGCCGC
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GCGCCTGGCCGCCTG
CGCCTGGCCGCCTGC
GCCTGGCCGCCTGCG
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TGGCCGCCTGCGGGC
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CTGCGGGCCCCCGCC
TGCGGGCCCCCGCCG
GCGGGCCCCCGCCGG

CGGGCCCCCGCCGGT
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CGCCGCGGTGGCCGC
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CCGCGGTGGCCGCAG
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GCCATGCGCGGAGCT
CCATGCGCGGAGCTC
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TGCGCGGAGCTCGTC
GCGCGGAGCTCGTCC
CGCGGAGCTCGTCCG
GCGGAGCTCGTCCGG
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CAGGTTGCAGACAAT
AGGTTGCAGACAATG
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AGACAATGGCGATGA
GACAATGGCGATGAC
ACAATGGCGATGACC
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AATGGCGATGACCAC
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CACTCAGAAGGAGGC
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CTCAGAAGGAGGCCT
TCAGAAGGAGGCCTG
CAGAAGGAGGCCTGG
AGAAGGAGGCCTGGT
GAAGGAGGCCTGGTG
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AGGAGGCCTGGTGGA
GGAGGCCTGGTGGAG
GAGGCCTGGTGGAGA
AGGCCTGGTGGAGAA
GGCCTGGTGGAGAAC
GCCTGGTGGAGAACC
CCTGGTGGAGAACCA
CTGGTGGAGAACCAC
TGGTGGAGAACCACG
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GAGAACCACGTGGAC
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GAACCACGTGGACAG
AACCACGTGGACAGC
ACCACGTGGACAGCA
CCACGTGGACAGCAC
CACGTGGACAGCACC
ACGTGGACAGCACCA
CGTGGACAGCACCAT
GTGGACAGCACCATG
TGGACAGCACCATGA
GGACAGCACCATGAA
GACAGCACCATGAAC
ACAGCACCATGAACA
CAGCACCATGAACAT
AGCACCATGAACATG
GCACCATGAACATGT

CACCATGAACATGTT
ACCATGAACATGTTG
CCATGAACATGTTGG
CATGAACATGTTGGG
ATGAACATGTTGGGC
TGAACATGTTGGGCG
GAACATGTTGGGCGG
AACATGTTGGGCGGG
ACATGTTGGGCGGGG
CATGTTGGGCGGGGG
ATGTTGGGCGGGGGA
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GTTGGGCGGGGGAGG
TTGGGCGGGGGAGGC
TGGGCGGGGGAGGCA
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GCAGTGCTGGCCGGA
CAGTGCTGGCCGGAA
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GTGCTGGCCGGAAGC
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CAAGTCGGGTATGAA
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GTCGGGTATGAAGGA
TCGGGTATGAAGGAG
CGGGTATGAAGGAGC
GGGTATGAAGGAGCT
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AAGGAGCTGGCCGTG
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TCCGGGAGAAGGTCA
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GGGAGAAGGTCACTG
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GAGAAGGTCACTGAG
AGAAGGTCACTGAGC
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GGTCACTGAGCAGCA
GTCACTGAGCAGCAC
TCACTGAGCAGCACC
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GGGCAAGGGTGGCAA

GGCAAGGGTGGCAAG
GCAAGGGTGGCAAGC
CAAGGGTGGCAAGCA
AAGGGTGGCAAGCAT
AGGGTGGCAAGCATC
GGGTGGCAAGCATCA
GGTGGCAAGCATCAC
GTGGCAAGCATCACC
TGGCAAGCATCACCT
GGCAAGCATCACCTT
GCAAGCATCACCTTG
CAAGCATCACCTTGG
AAGCATCACCTTGGC
AGCATCACCTTGGCC
GCATCACCTTGGCCT
CATCACCTTGGCCTG
ATCACCTTGGCCTGG
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GGAGGAGCCCAAGAA
GAGGAGCCCAAGAAG
AGGAGCCCAAGAAGC
GGAGCCCAAGAAGCT
GAGCCCAAGAAGCTG
AGCCCAAGAAGCTGC
GCCCAAGAAGCTGCG
CCCAAGAAGCTGCGA
CCAAGAAGCTGCGAC
CAAGAAGCTGCGACC
AAGAAGCTGCGACCA
AGAAGCTGCGACCAC
GAAGCTGCGACCACC
AAGCTGCGACCACCC
AGCTGCGACCACCCC
GCTGCGACCACCCCC
CTGCGACCACCCCCT
TGCGACCACCCCCTG
GCGACCACCCCCTGC
CGACCACCCCCTGCC
GACCACCCCCTGCCA
ACCACCCCCTGCCAG
CCACCCCCTGCCAGG
CACCCCCTGCCAGGA
ACCCCCTGCCAGGAC
CCCCCTGCCAGGACT
CCCCTGCCAGGACTC
CCCTGCCAGGACTCC
CCTGCCAGGACTCCC
CTGCCAGGACTCCCT
TGCCAGGACTCCCTG
GCCAGGACTCCCTGC
CCAGGACTCCCTGCC
CAGGACTCCCTGCCA
AGGACTCCCTGCCAA
GGACTCCCTGCCAAC
GACTCCCTGCCAACA
ACTCCCTGCCAACAG
CTCCCTGCCAACAGG
TCCCTGCCAACAGGA
CCCTGCCAACAGGAA
CCTGCCAACAGGAAC
CTGCCAACAGGAACT
TGCCAACAGGAACTG
GCCAACAGGAACTGG
CCAACAGGAACTGGA
CAACAGGAACTGGAC
AACAGGAACTGGACC
ACAGGAACTGGACCA
CAGGAACTGGACCAG
AGGAACTGGACCAGG
GGAACTGGACCAGGT
GAACTGGACCAGGTC
AACTGGACCAGGTCC
ACTGGACCAGGTCCT
CTGGACCAGGTCCTG
TGGACCAGGTCCTGG
GGACCAGGTCCTGGA
GACCAGGTCCTGGAG
ACCAGGTCCTGGAGC
CCAGGTCCTGGAGCG
CAGGTCCTGGAGCGG
AGGTCCTGGAGCGGA
GGTCCTGGAGCGGAT
GTCCTGGAGCGGATC
TCCTGGAGCGGATCT
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CTGGAGCGGATCTCC
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GGCCCTCTGGAGCAC
GCCCTCTGGAGCACC
CCCTCTGGAGCACCT
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TGGAGCACCTCTACT
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GCACCTCTACTCCCT
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ACCTCTACTCCCTGC
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CTCTACTCCCTGCAC
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CTACTCCCTGCACAT
TACTCCCTGCACATC
ACTCCCTGCACATCC
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CTGCACATCCCCAAC
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GCACATCCCCAACTG
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ACATCCCCAACTGTG
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TCCCCAACTGTGACA
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CCAACTGTGACAAGC
CAACTGTGACAAGCA
AACTGTGACAAGCAT
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CTGTGACAAGCATGG
TGTGACAAGCATGGC
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GACAAGCATGGCCTG
ACAAGCATGGCCTGT
CAAGCATGGCCTGTA
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AGTGCAAGATGTCTC
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TGCAAGATGTCTCTG
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CAAGATGTCTCTGAA
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TGTCTCTGAACGGGC
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TCTCTGAACGGGCAG
CTCTGAACGGGCAGC
TCTGAACGGGCAGCG
CTGAACGGGCAGCGT
TGAACGGGCAGCGTG
GAACGGGCAGCGTGG
AACGGGCAGCGTGGG
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AACACCGGGAAGCTG
ACACCGGGAAGCTGA
CACCGGGAAGCTGAT
ACCGGGAAGCTGATC
CCGGGAAGCTGATCC
CGGGAAGCTGATCCA
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GGACCCCGAGTGTCA
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ACCCCGAGTGTCATC
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CAGGAGGCTTGCGGG
AGGAGGCTTGCGGGG
GGAGGCTTGCGGGGT

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GGCTTGCGGGGTGCA	CGCCCCTGCCCCCCG	GGTGGGTGCTGGAGG
GCTTGCGGGGTGCAC	GCCCCTGCCCCCCGC	GTGGGTGCTGGAGGA
CTTGCGGGGTGCACA	CCCCTGCCCCCCGCC	TGGGTGCTGGAGGAT
TTGCGGGGTGCACAC	CCCTGCCCCCCGCCC	GGGTGCTGGAGGATT
TGCGGGGTGCACACC	CCTGCCCCCCGCCCC	GGTGCTGGAGGATTT
GCGGGGTGCACACCC	CTGCCCCCCGCCCCT	GTGCTGGAGGATTTT
CGGGGTGCACACCCA	TGCCCCCCGCCCCTC	TGCTGGAGGATTTTC
GGGGTGCACACCCAG	GCCCCCCGCCCCTCT	GCTGGAGGATTTTCC
GGGTGCACACCCAGC	CCCCCCGCCCCTCTC	CTGGAGGATTTTCCA
GGTGCACACCCAGCG	CCCCCGCCCCTCTCC	TGGAGGATTTTCCAG
GTGCACACCCAGCGG	CCCCGCCCCTCTCCA	GGAGGATTTTCCAGT
TGCACACCCAGCGGA	CCCGCCCCTCTCCAA	GAGGATTTTCCAGTT
GCACACCCAGCGGAT	CCGCCCCTCTCCAAA	AGGATTTTCCAGTTC
CACACCCAGCGGATG	CGCCCCTCTCCAAAC	GGATTTTCCAGTTCT
ACACCCAGCGGATGC	GCCCCTCTCCAAACA	GATTTTCCAGTTCTG
CACCCAGCGGATGCA	CCCCTCTCCAAACAC	ATTTTCCAGTTCTGA
ACCCAGCGGATGCAG	CCCTCTCCAAACACC	TTTTCCAGTTCTGAC
CCCAGCGGATGCAGT	CCTCTCCAAACACCG	TTTCCAGTTCTGACA
CCAGCGGATGCAGTA	CTCTCCAAACACCGG	TTCCAGTTCTGACAC
CAGCGGATGCAGTAG	TCTCCAAACACCGGC	TCCAGTTCTGACACA
AGCGGATGCAGTAGA	CTCCAAACACCGGCA	CCAGTTCTGACACAC
GCGGATGCAGTAGAC	TCCAAACACCGGCAG	CAGTTCTGACACACG
CGGATGCAGTAGACC	CCAAACACCGGCAGA	AGTTCTGACACACGT
GGATGCAGTAGACCG	CAAACACCGGCAGAA	GTTCTGACACACGTA
GATGCAGTAGACCGC	AAACACCGGCAGAAA	TTCTGACACACGTAT
ATGCAGTAGACCGCA	AACACCGGCAGAAAA	TCTGACACACGTATT
TGCAGTAGACCGCAG	ACACCGGCAGAAAAC	CTGACACACGTATTT
GCAGTAGACCGCAGC	CACCGGCAGAAAACG	TGACACACGTATTTA
CAGTAGACCGCAGCC	ACCGGCAGAAAACGG	GACACACGTATTTAT
AGTAGACCGCAGCCA	CCGGCAGAAAACGGA	ACACACGTATTTATA
GTAGACCGCAGCCAG	CGGCAGAAAACGGAG	CACACGTATTTATAT
TAGACCGCAGCCAGC	GGCAGAAAACGGAGA	ACACGTATTTATATT
AGACCGCAGCCAGCC	GCAGAAAACGGAGAG	CACGTATTTATATTT
GACCGCAGCCAGCCG	CAGAAAACGGAGAGT	ACGTATTTATATTTG
ACCGCAGCCAGCCGG	AGAAAACGGAGAGTG	CGTATTTATATTTGG
CCGCAGCCAGCCGGT	GAAAACGGAGAGTGC	GTATTTATATTTGGA
CGCAGCCAGCCGGTG	AAAACGGAGAGTGCT	TATTTATATTTGGAA
GCAGCCAGCCGGTGC	AAACGGAGAGTGCTT	ATTTATATTTGGAAA
CAGCCAGCCGGTGCC	AACGGAGAGTGCTTG	TTTATATTTGGAAAG
AGCCAGCCGGTGCCT	ACGGAGAGTGCTTGG	TTATATTTGGAAAGA
GCCAGCCGGTGCCTG	CGGAGAGTGCTTGGG	TATATTTGGAAAGAG
CCAGCCGGTGCCTGG	GGAGAGTGCTTGGGT	ATATTTGGAAAGAGA
CAGCCGGTGCCTGGC	GAGAGTGCTTGGGTG	TATTTGGAAAGAGAC
AGCCGGTGCCTGGCG	AGAGTGCTTGGGTGG	ATTTGGAAAGAGACC
GCCGGTGCCTGGCGC	GAGTGCTTGGGTGGT	TTTGGAAAGAGACCA
CCGGTGCCTGGCGCC	AGTGCTTGGGTGGTG	TTGGAAAGAGACCAG
CGGTGCCTGGCGCCC	GTGCTTGGGTGGTGG	TGGAAAGAGACCAGC
GGTGCCTGGCGCCCC	TGCTTGGGTGGTGGG	GGAAAGAGACCAGCA
GTGCCTGGCGCCCCT	GCTTGGGTGGTGGGT	GAAAGAGACCAGCAC
TGCCTGGCGCCCCTG	CTTGGGTGGTGGGTG	AAAGAGACCAGCACC
GCCTGGCGCCCCTGC	TTGGGTGGTGGGTGC	AAGAGACCAGCACCG
CCTGGCGCCCCTGCC	TGGGTGGTGGGTGCT	AGAGACCAGCACCGA
CTGGCGCCCCTGCCC	GGGTGGTGGGTGCTG	GAGACCAGCACCGAG
TGGCGCCCCTGCCCC	GGTGGTGGGTGCTGG	AGACCAGCACCGAGC

GACCAGCACCGAGCT	CACCTGCTCCTTCTT	GGGTACAGGTTTGGG
ACCAGCACCGAGCTC	ACCTGCTCCTTCTTG	GGTACAGGTTTGGGG
CCAGCACCGAGCTCG	CCTGCTCCTTCTTGC	GTACAGGTTTGGGGA
CAGCACCGAGCTCGG	CTGCTCCTTCTTGCT	TACAGGTTTGGGGAG
AGCACCGAGCTCGGC	TGCTCCTTCTTGCTT	ACAGGTTTGGGGAGG
GCACCGAGCTCGGCA	GCTCCTTCTTGCTTT	CAGGTTTGGGGAGGG
CACCGAGCTCGGCAC	CTCCTTCTTGCTTTC	AGGTTTGGGGAGGGG
ACCGAGCTCGGCACC	TCCTTCTTGCTTTCC	GGTTTGGGGAGGGGG
CCGAGCTCGGCACCT	CCTTCTTGCTTTCCC	GTTTGGGGAGGGGGA
CGAGCTCGGCACCTC	CTTCTTGCTTTCCCC	TTTGGGGAGGGGGAA
GAGCTCGGCACCTCC	TTCTTGCTTTCCCCG	TTGGGGAGGGGGAAG
AGCTCGGCACCTCCC	TCTTGCTTTCCCCGG	TGGGGAGGGGGAAGA
GCTCGGCACCTCCCC	CTTGCTTTCCCCGGG	GGGGAGGGGGAAGAG
CTCGGCACCTCCCCG	TTGCTTTCCCCGGGG	GGGAGGGGGAAGAGA
TCGGCACCTCCCCGG	TGCTTTCCCCGGGGG	GGAGGGGGAAGAGAA
CGGCACCTCCCCGGC	GCTTTCCCCGGGGGA	GAGGGGGAAGAGAAA
GGCACCTCCCCGGCC	CTTTCCCCGGGGGAG	AGGGGGAAGAGAAAT
GCACCTCCCCGGCCT	TTTCCCCGGGGGAGG	GGGGGAAGAGAAATT
CACCTCCCCGGCCTC	TTCCCCGGGGGAGGA	GGGGAAGAGAAATTT
ACCTCCCCGGCCTCT	TCCCCGGGGGAGGAA	GGGAAGAGAAATTTT
CCTCCCCGGCCTCTC	CCCCGGGGGAGGAAG	GGAAGAGAAATTTTT
CTCCCCGGCCTCTCT	CCCGGGGGAGGAAGG	GAAGAGAAATTTTTA
TCCCCGGCCTCTCTC	CCGGGGGAGGAAGGG	AAGAGAAATTTTTAT
CCCCGGCCTCTCTCT	CGGGGGAGGAAGGGG	AGAGAAATTTTTATT
CCCGGCCTCTCTCTT	GGGGGAGGAAGGGGG	GAGAAATTTTTATTT
CCGGCCTCTCTCTTC	GGGGAGGAAGGGGGT	AGAAATTTTTATTTT
CGGCCTCTCTCTTCC	GGGAGGAAGGGGGTT	GAAATTTTTATTTTT
GGCCTCTCTCTTCCC	GGAGGAAGGGGGTTG	AAATTTTTATTTTTG
GCCTCTCTCTTCCCA	GAGGAAGGGGGTTGT	AATTTTTATTTTTGA
CCTCTCTCTTCCCAG	AGGAAGGGGGTTGTG	ATTTTTATTTTTGAA
CTCTCTCTTCCCAGC	GGAAGGGGGTTGTGG	TTTTTATTTTTGAAC
TCTCTCTTCCCAGCT	GAAGGGGGTTGTGGT	TTTTATTTTTGAACC
CTCTCTTCCCAGCTG	AAGGGGGTTGTGGTC	TTTATTTTTGAACCC
TCTCTTCCCAGCTGC	AGGGGGTTGTGGTCG	TTATTTTTGAACCCC
CTCTTCCCAGCTGCA	GGGGGTTGTGGTCGG	TATTTTTGAACCCCT
TCTTCCCAGCTGCAG	GGGGTTGTGGTCGGG	ATTTTTGAACCCCTG
CTTCCCAGCTGCAGA	GGGTTGTGGTCGGGG	TTTTTGAACCCCTGT
TTCCCAGCTGCAGAT	GGTTGTGGTCGGGGA	TTTTGAACCCCTGTG
TCCCAGCTGCAGATG	GTTGTGGTCGGGGAG	TTTGAACCCCTGTGT
CCCAGCTGCAGATGC	TTGTGGTCGGGGAGC	TTGAACCCCTGTGTC
CCAGCTGCAGATGCC	TGTGGTCGGGGAGCT	TGAACCCCTGTGTCC
CAGCTGCAGATGCCA	GTGGTCGGGGAGCTG	GAACCCCTGTGTCCC
AGCTGCAGATGCCAC	TGGTCGGGGAGCTGG	AACCCCTGTGTCCCT
GCTGCAGATGCCACA	GGTCGGGGAGCTGGG	ACCCCTGTGTCCCTT
CTGCAGATGCCACAC	GTCGGGGAGCTGGGG	CCCCTGTGTCCCTTT
TGCAGATGCCACACC	TCGGGGAGCTGGGGT	CCCTGTGTCCCTTTT
GCAGATGCCACACCT	CGGGGAGCTGGGGTA	CCTGTGTCCCTTTTG
CAGATGCCACACCTG	GGGGAGCTGGGGTAC	CTGTGTCCCTTTTGC
AGATGCCACACCTGC	GGGAGCTGGGGTACA	TGTGTCCCTTTTGCA
GATGCCACACCTGCT	GGAGCTGGGGTACAG	GTGTCCCTTTTGCAT
ATGCCACACCTGCTC	GAGCTGGGGTACAGG	TGTCCCTTTTGCATA
TGCCACACCTGCTCC	AGCTGGGGTACAGGT	GTCCCTTTTGCATAA
GCCACACCTGCTCCT	GCTGGGGTACAGGTT	TCCCTTTTGCATAAG
CCACACCTGCTCCTT	CTGGGGTACAGGTTT	CCCTTTTGCATAAGA
CACACCTGCTCCTTC	TGGGGTACAGGTTTG	CCTTTTGCATAAGAT
ACACCTGCTCCTTCT	GGGGTACAGGTTTGG	CTTTTGCATAAGATT

TTTTGCATAAGATTA
TTTGCATAAGATTAA
TTGCATAAGATTAAA
TGCATAAGATTAAAG
GCATAAGATTAAAGG
CATAAGATTAAAGGA
ATAAGATTAAAGGAA
TAAGATTAAAGGAAG
AAGATTAAAGGAAGG
AGATTAAAGGAAGGA
GATTAAAGGAAGGAA
ATTAAAGGAAGGAAA
TTAAAGGAAGGAAAA
TAAAGGAAGGAAAAG
AAAGGAAGGAAAAGT

EXAMPLE 7

ANTISENSE OLIGONUCLEOTIDES OF IGFBP3

Antisense oligonucleotides to IGFBP3 may be selected from molecules capable of interacting with one or more of the following sense oligonucleotides:

CTCAGCGCCCAGCCG
TCAGCGCCCAGCCGC
CAGCGCCCAGCCGCT
AGCGCCCAGCCGCTT
GCGCCCAGCCGCTTC
CGCCCAGCCGCTTCC
GCCCAGCCGCTTCCT
CCCAGCCGCTTCCTG
CCAGCCGCTTCCTGC
CAGCCGCTTCCTGCC
AGCCGCTTCCTGCCT
GCCGCTTCCTGCCTG
CCGCTTCCTGCCTGG
CGCTTCCTGCCTGGA
GCTTCCTGCCTGGAT
CTTCCTGCCTGGATT
TTCCTGCCTGGATTC
TCCTGCCTGGATTCC
CCTGCCTGGATTCCA
CTGCCTGGATTCCAC
TGCCTGGATTCCACA
GCCTGGATTCCACAG
CCTGGATTCCACAGC
CTGGATTCCACAGCT
TGGATTCCACAGCTT
GGATTCCACAGCTTC
GATTCCACAGCTTCG
ATTCCACAGCTTCGC
TTCCACAGCTTCGCG
TCCACAGCTTCGCGC
CCACAGCTTCGCGCC
CACAGCTTCGCGCCG
ACAGCTTCGCGCCGT
CAGCTTCGCGCCGTG
AGCTTCGCGCCGTGT
GCTTCGCGCCGTGTA
CTTCGCGCCGTGTAC
TTCGCGCCGTGTACT
TCGCGCCGTGTACTG
CGCGCCGTGTACTGT
GCGCCGTGTACTGTC
CGCCGTGTACTGTCG
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CCGTGTACTGTCGCC
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TGTCGCCCCATCCCT
GTCGCCCCATCCCTG
TCGCCCCATCCCTGC
CGCCCCATCCCTGCG
GCCCCATCCCTGCGC
CCCCATCCCTGCGCG
CCCATCCCTGCGCGC
CCATCCCTGCGCGCC
CATCCCTGCGCGCCC
ATCCCTGCGCGCCCA
TCCCTGCGCGCCCAG
CCCTGCGCGCCCAGC
CCTGCGCGCCCAGCC
CTGCGCGCCCAGCCT
TGCGCGCCCAGCCTG
GCGCGCCCAGCCTGC
CGCGCCCAGCCTGCC
GCGCCCAGCCTGCCA
CGCCCAGCCTGCCAA
GCCCAGCCTGCCAAG
CCCAGCCTGCCAAGC
CCAGCCTGCCAAGCA
CAGCCTGCCAAGCAG
AGCCTGCCAAGCAGC
GCCTGCCAAGCAGCG
CCTGCCAAGCAGCGT
CTGCCAAGCAGCGTG
TGCCAAGCAGCGTGC
GCCAAGCAGCGTGCC
CCAAGCAGCGTGCCC
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AGCAGCGTGCCCCGG
GCAGCGTGCCCCGGT
CAGCGTGCCCCGGTT
AGCGTGCCCCGGTTG
GCGTGCCCCGGTTGC
CGTGCCCCGGTTGCA
GTGCCCCGGTTGCAG

TGCCCCGGTTGCAGG
GCCCCGGTTGCAGGC
CCCCGGTTGCAGGCG
CCCGGTTGCAGGCGT
CCGGTTGCAGGCGTC
CGGTTGCAGGCGTCA
GGTTGCAGGCGTCAT
GTTGCAGGCGTCATG
TTGCAGGCGTCATGC
TGCAGGCGTCATGCA
GCAGGCGTCATGCAG
CAGGCGTCATGCAGC
AGGCGTCATGCAGCG
GGCGTCATGCAGCGG
GCGTCATGCAGCGGG
CGTCATGCAGCGGGC
GTCATGCAGCGGGCG
TCATGCAGCGGGCGC
CATGCAGCGGGCGCG
ATGCAGCGGGCGCGA
TGCAGCGGGCGCGAC
GCAGCGGGCGCGACC
CAGCGGGCGCGACCC
AGCGGGCGCGACCCA
GCGGGCGCGACCCAC
CGGGCGCGACCCACG
GGGCGCGACCCACGC
GGCGCGACCCACGCT
GCGCGACCCACGCTC
CGCGACCCACGCTCT
GCGACCCACGCTCTG
CGACCCACGCTCTGG
GACCCACGCTCTGGG
ACCCACGCTCTGGGC
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CCACGCTCTGGGCCG
CACGCTCTGGGCCGC
ACGCTCTGGGCCGCT
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CCGCTGCGCTGACTC
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CTCCGCGGGCCGCCG
TCCGCGGGCCGCCGG
CCGCGGGCCGCCGGT
CGCGGGCCGCCGGTG
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CGCGAGCTCGGGGGG
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CGAGCTCGGGGGGCT
GAGCTCGGGGGGCTT
AGCTCGGGGGGCTTG
GCTCGGGGGGCTTGG
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TCGGGGGGCTTGGGT
CGGGGGGCTTGGGTC
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GGGGCTTGGGTCCCG
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CCGTGGTGCGCTGCG
CGTGGTGCGCTGCGA
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GGTGCGCTGCGAGCC
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GCGCTGCGAGCCGTG
CGCTGCGAGCCGTGC
GCTGCGAGCCGTGCG
CTGCGAGCCGTGCGA
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GAGCCGTGCGACGCG
AGCCGTGCGACGCGC
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CGTGCGACGCGCGTG
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TGCGACGCGCGTGCA
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CGACGCGCGTGCACT
GACGCGCGTGCACTG
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CGCGCGTGCACTGGC
GCGCGTGCACTGGCC
CGCGTGCACTGGCCC
GCGTGCACTGGCCCA
CGTGCACTGGCCCAG
GTGCACTGGCCCAGT
TGCACTGGCCCAGTG
GCACTGGCCCAGTGC
CACTGGCCCAGTGCG
ACTGGCCCAGTGCGC
CTGGCCCAGTGCGCG
TGGCCCAGTGCGCGC
GGCCCAGTGCGCGCC
GCCCAGTGCGCGCCT
CCCAGTGCGCGCCTC
CCAGTGCGCGCCTCC
CAGTGCGCGCCTCCG
AGTGCGCGCCTCCGC

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TGCGCGCCTCCGCCC
GCGCGCCTCCGCCCG
CGCGCCTCCGCCCGC
GCGCCTCCGCCCGCC
CGCCTCCGCCCGCCG
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CCTCCGCCCGCCGTG
CTCCGCCCGCCGTGT
TCCGCCCGCCGTGTG
CCGCCCGCCGTGTGC
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ATAGTAAGATCTATA
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CAACTGTGGCCATGA
AACTGTGGCCATGAC
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CTGTGGCCATGACTG
TGTGGCCATGACTGA
GTGGCCATGACTGAG
TGGCCATGACTGAGG
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GACTGAGGAAAGGAG
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TCAGGCTCAGGGAGA
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AGGCTCAGGGAGACT
GGCTCAGGGAGACTC

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CTCAGGGAGACTCTG
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CAGGGAGACTCTGCC
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GACACACGCTGCATA
ACACACGCTGCATAG
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ACACGCTGCATAGAG
CACGCTGCATAGAGC
ACGCTGCATAGAGCT
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AGCTCTCCTTGAAAA
GCTCTCCTTGAAAAC

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CCTTGAAAACAGAGG
CTTGAAAACAGAGGG
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TTTTAACTTTTTGGG
TTTAACTTTTTGGGG
TTAACTTTTTGGGGG
TAACTTTTTGGGGGG
AACTTTTTGGGGGGA
ACTTTTTGGGGGGAA
CTTTTTGGGGGGAAA
TTTTTGGGGGGAAAA
TTTTGGGGGGAAAAG
TTTGGGGGGAAAAGT
TTGGGGGGAAAAGTA
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GCAATGTATTTATAA
CAATGTATTTATAAA
AATGTATTTATAAAT
ATGTATTTATAAATA
TGTATTTATAAATAG
GTATTTATAAATAGT
TATTTATAAATAGTA

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TAAATAGTAAATAAA
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ATAGTAAATAAAGTT
TAGTAAATAAAGTTT
AGTAAATAAAGTTTT
GTAAATAAAGTTTTT
TAAATAAAGTTTTTA
AAATAAAGTTTTTAC
AATAAAGTTTTTACC
ATAAAGTTTTTACCA
TAAAGTTTTTACCAT
AAAGTTTTTACCATT

EXAMPLE 8

ANTISENSE OLIGONUCLEOTIDES OF IGF-I RECEPTOR

Antisense oligonucleotides to IGF-I may be selected from molecules capable of interacting with one or more of the following sense oligonucleotides:

TTTTTTTTTTTTTTG
TTTTTTTTTTTTTGA
TTTTTTTTTTTTGAG
TTTTTTTTTTTGAGA
TTTTTTTTTTGAGAA
TTTTTTTTTGAGAAA
TTTTTTTTGAGAAAG
TTTTTTTGAGAAAGG
TTTTTTGAGAAAGGG
TTTTTGAGAAAGGGA
TTTTGAGAAAGGGAA
TTTGAGAAAGGGAAT
TTGAGAAAGGGAATT
TGAGAAAGGGAATTT
GAGAAAGGGAATTTC
AGAAAGGGAATTTCA
GAAAGGGAATTTCAT
AAAGGGAATTTCATC
AAGGGAATTTCATCC
AGGGAATTTCATCCC
GGGAATTTCATCCCA
GGAATTTCATCCCAA
GAATTTCATCCCAAA
AATTTCATCCCAAAT
ATTTCATCCCAAATA
TTTCATCCCAAATAA
TTCATCCCAAATAAA
TCATCCCAAATAAAA
CATCCCAAATAAAAG
ATCCCAAATAAAAGG
TCCCAAATAAAAGGA
CCCAAATAAAAGGAA
CCAAATAAAAGGAAT
CAAATAAAAGGAATG
AAATAAAAGGAATGA
AATAAAAGGAATGAA
ATAAAAGGAATGAAG
TAAAAGGAATGAAGT
AAAAGGAATGAAGTC
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AGGAATGAAGTCTGG
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GAATGAAGTCTGGCT
AATGAAGTCTGGCTC
ATGAAGTCTGGCTCC
TGAAGTCTGGCTCCG
GAAGTCTGGCTCCGG
AAGTCTGGCTCCGGA
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GTCTGGCTCCGGAGG
TCTGGCTCCGGAGGA
CTGGCTCCGGAGGAG
TGGCTCCGGAGGAGG
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CTCCGGAGGAGGGTC
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GGTCCCCGACCTCGC
GTCCCCGACCTCGCT
TCCCCGACCTCGCTG
CCCCGACCTCGCTGT
CCCGACCTCGCTGTG
CCGACCTCGCTGTGG
CGACCTCGCTGTGGG
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ACCTCGCTGTGGGGG
CCTCGCTGTGGGGGC
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CGCTGTGGGGGCTCC
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GGCTCCTGTTTCTCT
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CTCCTGTTTCTCTCC
TCCTGTTTCTCTCCG
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TGTTTCTCTCCGCCG
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TCTCTCCGCCGCGCT
CTCTCCGCCGCGCTC
TCTCCGCCGCGCTCT
CTCCGCCGCGCTCTC
TCCGCCGCGCTCTCG
CCGCCGCGCTCTCGC
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CCGCGCTCTCGCTCT
CGCGCTCTCGCTCTG
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CGACGAGTGGAGAAA
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CTGGAGAACTGCACG
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ATCAGGATTGAGAAA
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TTGAGAAAAATGCTG

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AGAAAAATGCTGACC
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AAAAATGCTGACCTC
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AAATGCTGACCTCTG
AATGCTGACCTCTGT
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TGCTGACCTCTGTTA
GCTGACCTCTGTTAC
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TGACCTCTGTTACCT
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CCTCTGTTACCTCTC
CTCTGTTACCTCTCC
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CTGTTACCTCTCCAC
TGTTACCTCTCCACT
GTTACCTCTCCACTG
TTACCTCTCCACTGT
TACCTCTCCACTGTG
ACCTCTCCACTGTGG
CCTCTCCACTGTGGA
CTCTCCACTGTGGAC
TCTCCACTGTGGACT
CTCCACTGTGGACTG
TCCACTGTGGACTGG
CCACTGTGGACTGGT
CACTGTGGACTGGTC
ACTGTGGACTGGTCC
CTGTGGACTGGTCCC
TGTGGACTGGTCCCT
GTGGACTGGTCCCTG
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GATGCGGTGTCCAAT

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GCGGTGTCCAATAAC
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GTGTCCAATAACTAC
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EXAMPLE 9

INHIBITION OF IGF-I BINDING BY ANTISENSE OLIGONUCLEOTIDES TO IGF-I RECEPTOR

Sub-confluent HaCaT cells were treated as described above with phosphorothioate oligonucleotides IGFR.AS (antisense: 5′-ATCTCTCCGCTTCCTTTC-3′; [SEQ ID NO. 10]; ref 13) and IGFR.S (sense control: 5′-GAAAGGAAGCGGAGAGAT-3′; [SEQ ID NO. 11]; ref 13) IGF-I binding to the cell monolayers was then measured as ^{125}I-IGF-I.

EXAMPLE 10

INHIBITION OF IGFBP-3 PRODUCTION USING ANTISENSE OLIGONUCLEOTIDES

The results of this experiment are shown in Figures 7 and 8.

HaCaT cells were initially plated in DMEM with 10% v/v serum, then AS oligo experiments were performed in complete "Keratinocyte-SFM" (Gibco) to exclude the influence of exogenous IGFBPs. Oligos were synthesised as phosphorothioate (nuclease-resistant) derivatives (Bresatec, South Australia) and were as follows: antisense: AS2, 5′-GCGCCCGCTGCATGACGCCTGCAAC-3′ (IGFBP-3 start codon); controls:

AS2NS, 5′-CGGAGATGCCGCATGCCAGCGCAGG-3′; AS4, 5′-AGGCGGCTGACGGCACTA-3′; AS4NS, 5′-GACAGCGTCGGAGCGATC-3′; IGFRAS, 5′-ATCTCTCCGCTTCCTTTC-3′; IGFRS, 5′-GAAAGGAAGCGGAGAGAT-3′. Oligos to IGFBP-3 were based on the published sequence of Spratt *et al* [12]. AS oligos were added to HaCaT monolayers in 0.5ml medium in 24-well plates at the concentrations and addition frequencies indicated. IGFBP-3 measured in cell-conditioned medium using a dot-blot assay, adapted from the Western ligand blot method of Hossenlopp *et al* [11], in which 100µl of conditioned medium was applied to nitrocellulose filters with a vacuum dot-blot apparatus. After drying the membranes at 37°C, relative amounts of IGFBP are determined by ^{125}I-IGF-I-binding, autoradiography and computerised imaging densitometry. Triplicate wells (except in Figure 7, where duplicate wells were measured as shown) were analysed and corrected for changes in cell number per well. Relative cell number per well was determined using an amido black dye method, developed specifically for cultured monolayers of HaCaT cells (14). Cell numbers differed by less than 10% after treatment. For oligos to the IGF receptor, receptor quantitation in intact HaCaT monolayers was by overnight incubation with ^{125}I-IGF-I (30,000cpm/well) at 4°C.

EXAMPLE 11

INHIBITION OF IGFBP-2 PRODUCTION USING RIBOZYMES

Experiments involving ribozymes are generally conducted as described in Internaitonal Patent Application No. WO 89/05852 and in Haselhoff and Gerlach [8]. Ribozymes are constructed with a hybridising region which is complementary in nucleotide sequence to at least part of a target RNA which, in this case, encodes IGFBP-2. Activity of ribozymes is measurable on, for example, Northern blots or using animal models such as in the nude mouse model (15; 16) or the "flaky skin" mouse model (17; 18).

EXAMPLE 12

INHIBITION OF IGFBP-3 PRODUCTION USING RIBOZYMES

The methods described in Example 11 are used for the screening of ribozymes which inhibit IGFBP-3 production. The activity of the ribozymes is determined as in Example 11.

EXAMPLE 13

INHIBITION OF IGF-1 PRODUCTION USING RIBOZYMES

The methods described in Example 11 are used for the screening of ribozymes which inhibit IGF-1 production. The activity of the ribozymes is determined as in Example 11.

EXAMPLE 14

INHIBITION OF IGF-1 RECEPTOR PRODUCTION USING RIBOZYMES

The methods described in Example 11 are used for the screening of ribozymes which inhibit IGF-1 production. The activity of the ribozymes is determined as in Example 11.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT (countries other than US): ROYAL CHILDREN'S HOSPITAL RESEARCH FOUNDATION

(US only): George A WERTHER and Christopher J WRAIGHT

(ii) TITLE OF INVENTION: A METHOD FOR THE PROPHYLAXIS AND/OR TREATMENT OF PROLIFERATIVE AND/OR INFLAMMATORY SKIN DISORDERS

(iii) NUMBER OF SEQUENCES: 11

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(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 06-JUL-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PM6725/94
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(viii) ATTORNEY/AGENT INFORMATION:
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(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1433 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCGCTGCTGC TGCTGCTACT GGGCGCGAGT GGCGGCGGCG GCGGGGCGCG CGCGGAGGTG 240

CTGTTCCGCT GCCCGCCCTG CACACCCGAG CGCCTGGCCG CCTGCGGGCC CCCGCCGGTT 300

GCGCCGCCCG CCGCGGTGGC CGCAGTGGCC GGAGGCGCCC GCATGCCATG CGCGGAGCTC 360

GTCCGGGAGC CGGGCTGCGG CTGCTGCTCG GTGTGCGCCC GGCTGGAGGG CGAGGCGTGC 420

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CAGCGTGGGG AGTGCTGGTG TGTGAACCCC AACACCGGGA AGCTGATCCA GGGAGCCCCC 1020

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2474 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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CACCGAGCGC TGTGGCTCCG GCCTTCGCTG CCAGCCGTCG CCCGACGAGG CGCGACCGCT   420
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CACCCAGACT TCATGCGCCC GTGGAATGCT CACCACATGT TGGTCGAAGC GGCCGACCAC  1320
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4989 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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TGGCCGACGA GTGGAGAAAT CTGCGGGCCA GGCATCGACA TCCGCAACGA CTATCAGCAG  180
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGGCGGCTC ACCTGGAGCT GGCG 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGGCGGCTGA CGGCACTA 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGGCGTCAT GCAGCGGGC 19

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGGAGATGCC GCATGCCAGC GCAGG 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACAGCGTCG GAGCGATC 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAAAGGAAGC GGAGAGAT 18

CLAIMS:

1. A method for ameliorating the effects of a proliferative and/or inflammatory skin disorder in a mammal, said method comprising contacting the proliferating and/or inflamed skin or skin capable of proliferation and/or inflammation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing growth factor mediated cell proliferation and/or inflammation.

2. A method according to claim 1 wherein cell proliferation and/or inflammation is mediated by at least one of insulin-like growth factor I (IGF-I), keratinocyte growth factor (KGF), transforming growth factor-α (TGFα), tumour necrosis factor-α (TNFα), interleukin (IL) -1 (IL-1), IL-4, IL-6, IL-8 and/or basic fibroblast growth factor (bFGF).

3. A method according to claim 2 wherein cell proliferation and/or inflammation is mediated by IGF-I.

4. A method according to claim 1 wherein the nucleic acid molecule inhibits or otherwise reduces IGF-I mediated cell proliferation and/or inflammation.

5. A method according to claim 1 wherein the proliferative or inflammatory skin disorder is psoriasis, ichthyosis, pityriasis, rubra, pilaris, serborrhoea, keloids, keratosis, neoplasias, scleroderma, warts, benign growths or cancers of the skin.

6. A method according to claim 5 wherein the skin condition is psoriasis.

7. A method according to claim 1 or 4 or 6 wherein the mammal is a human.

8. A method according to claim 1 or 4 or 6 wherein the nucleic acid molecule is capable of inhibiting, reducing or otherwise interfering with IGF-I-interaction with its receptor.

9. A method according to claim 8 wherein the nucleic acid molecule is an antisense molecule capable of reducing expression of a gene encoding IGF-I, IGF-I-receptor or an IGF binding protein (IGFBP).

10. A method according to claim 9 wherein the nucleic acid molecule is an antisense molecule capable of reducing expression of a gene encoding IGFBP-2, -3, -4, -5 or -6.

11. A method according to claim 10 wherein the nucleic acid molecule is an antisense molecule capable of reducing expression of a gene encoding IGFBP-2 or IGFBP-3.

12. A method according to claim 11 wherein the antisense molecule is at least about 15 nucleotides in length and is capable of interacting with at least one sequence selected from the list set forth in Example 6 or Example 7.

13. A method according to claim 11 wherein the antisense molecule comprises the nucleotide sequence:

5'-ATCTCTCCGCTTCCTTTC-3' [SEQ ID NO:10].

14. A nucleic acid molecule comprising at least about 10 nucleotides capable of hybridising to or forming a heteroduplex or otherwise interacting with an RNA molecule directed from a gene corresponding to a genomic form of SEQ ID NO:1 and/or SEQ ID NO:2 and which thereby reduces or inhibits translation of said RNA molecule.

15. A nucleic acid molecule according to claim 14 wherein said molecule comprises at least about 15 nucleotides.

16. A nucleic acid molecule according to claim 15 wherein said molecule is capable of interacting with at least one nucleotide sequence selected from the list set forth in Example 6 and Example 7.

17. A nucleic acid molecule according to claim 15 or 16 comprising the nucleotide sequence:

5'-ATCTCTCCGCTTCCTTTC-3' [SEQ ID NO:10].

18. A method of ameliorating the effects of psoriasis, said method comprising contacting proliferating skin or skin capable of proliferation with an effective amount of one or more nucleic acid molecules or chemical analogues thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation wherein said one or more molecules comprises a polynucleotide capable of interacting with mRNA directed from an IGF-I gene, an IGF-I receptor gene or a gene encoding an IGFBP.

19. A method according to claim 18 wherein the IGFBP is IGFBP-2 or IGFBP-3.

20. A method according to claim 18 or 19 wherein the mammal is a human.

21. A method according to claim 20 wherein the nucleic acid molecule is capable of interacting with a nucleotide sequence selected from the list set forth in Example 6 or Example 7.

22. A method according to claim 18 wherein the nucleic acid molecule comprises the nucleotide sequence:

5'-ATCTCTCCGCTTCCTTTC-3' [SEQ ID NO:10].

23. A pharmaceutical composition for topical administration said composition comprising a nucleic acid molecule capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

24. A pharmaceutical composition according to claim 23 wherein the nucleic acid molecule is an antisense molecule to a gene encoding IGF-I, IGF-I-receptor or an IGFBP.

25. A pharmaceutical composition according to claim 24 wherein the nucleic acid molecule is capable of targeting a gene encoding IGFBP-2 and/or IGFBP-3.

26. A pharmaceutical composition according to claim 24 capable of interacting with at least one nucleotide sequence set forth in Example 6 or Example 7.

27. Use of a nucleic acid molecule in the manufacture of a medicament for the treatment of a proliferative and/or inflammatory skin disorder mediated by IGF-I.

28. Use according to claim 27 wherein the skin disorder is psoriasis.

29. A ribozyme comprising a hybridising region and a catalytic region wherein the hybridising region is capable of hybridising to at least part of a target mRNA sequence transcribed from a genomic gene corresponding to SEQ ID NO:1 or SEQ ID NO:2 wherein said catalytic domain is capable of cleaving said target mRNA sequence to reduce or inhibit IGF-I mediated cell proliferation or inflammation.

ABSTRACT

The present invention relates generally to a method for the prophylaxis and/or treatment of skin disorders, and in particular proliferative and/or inflammatory skin disorders, and to genetic molecules useful for same. The present invention is particularly directed to genetic molecules capable of modulating growth factor interaction with its receptor on epidermal keratinocytes to inhibit, reduce or otherwise decrease stimulation of this layer of cells. The present invention contemplates, in a most preferred embodiment, a method for the prophylaxis and/or treatment of psoriasis.

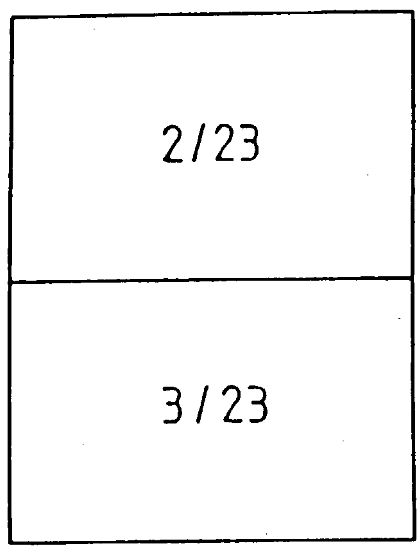

FIG. 1

1	ATTCGGGGCG	AGGGAGGAGG	AAGAAGCGGA	GGAGGCGGCT	CCCGCTCGCA
51	GGGCCGTGCA	CCTGCCCGCC	CGCCCGCTCG	CTCGCTCGCC	CGCCGCGCCG
101	CGCTGCCGAC	CGCCAGCATG	CTGCCGAGAG	TGGGCTGCCC	CGCGCTGCCG
151	CTGCCGCCGC	CGCCGCTGCT	GCCGCTGCTG	CCGCTGCTGC	TGCTGCTACT
201	GGGCGCGAGT	GGCGGCGGCG	GCGGGGCGCG	CGCGGAGGTG	CTGTTCCGCT
251	GCCCGCCCTG	CACACCCGAG	CGCCTGGCCG	CCTGCGGGCC	CCCGCCGGTT
301	GCGCCGCCCG	CCGCGGTGGC	CGCAGTGGCC	GGAGGCGCCC	GCATGCCATG
351	CGCGGAGCTC	GTCCGGGAGC	CGGGCTGCGG	CTGCTGCTCG	GTGTGCGCCC
401	GGCTGGAGGG	CGAGGCGTGC	GGCGTCTACA	CCCCGCGCTG	CGGCCAGGGG
451	CTGCGCTGCT	ATCCCCACCC	GGGCTCCGAG	CTGCCCCTGC	AGGCGCTGGT
501	CATGGGCGAG	GGCACTTGTG	AGAAGCGCCG	GGACGCCGAG	TATGGCGCCA
551	GCCCGGAGCA	GGTTGCAGAC	AATGGCGATG	ACCACTCAGA	AGGAGGCCTG
601	GTGGAGAACC	ACGTGGACAG	CACCATGAAC	ATGTTGGGCG	GGGGAGGCAG
651	TGCTGGCCGG	AAGCCCCTCA	AGTCGGGTAT	GAAGGAGCTG	GCCGTGTTCC
701	GGGAGAAGGT	CACTGAGCAG	CACCGGCAGA	TGGGCAAGGG	TGGCAAGCAT

FIG. 1A

751	CACCTTGGCC	TGGAGGAGCC	CAAGAAGCTG	CGACCACCCC	CTGCCAGGAC
801	TCCCTGCCAA	CAGGAACTGG	ACCAGGTCCT	GGAGCGGATC	TCCACCATGC
851	GCCTTCCGGA	TGAGCGGGGC	CCTCTGGAGC	ACCTCTACTC	CCTGCACATC
901	CCCAACTGTG	ACAAGCATGG	CCTGTACAAC	CTCAAACAGT	GCAAGATGTC
951	TCTGAACGGG	CAGCGTGGGG	AGTGCTGGTG	TGTGAACCCC	AACACCGGGA
1001	AGCTGATCCA	GGGAGCCCCC	ACCATCCGGG	GGGACCCCGA	GTGTCATCTC
1051	TTCTACAATG	AGCAGCAGGA	GGCTTGCGGG	GTGCACACCC	AGCGGATGCA
1101	GTAGACCGCA	GCCAGCCGGT	GCCTGGCGCC	CCTGCCCCCC	GCCCCTCTCC
1151	AAACACCGGC	AGAAAACGGA	GAGTGCTTGG	GTGGTGGGTG	CTGGAGGATT
1201	TTCCAGTTCT	GACACACGTA	TTTATATTTG	GAAAGAGACC	AGCACCGAGC
1251	TCGGCACCTC	CCCGGCCTCT	CTCTTCCCAG	CTGCAGATGC	CACACCTGCT
1301	CCTTCTTGCT	TTCCCCGGGG	GAGGAAGGGG	GTTGTGGTCG	GGGAGCTGGG
1351	GTACAGGTTT	GGGGAGGGGG	AAGAGAAATT	TTTATTTTTG	AACCCCTGTG
1401	TCCCTTTTGC	ATAAGATTAA	AGGAAGGAAA	AGT	

FIG. 1B

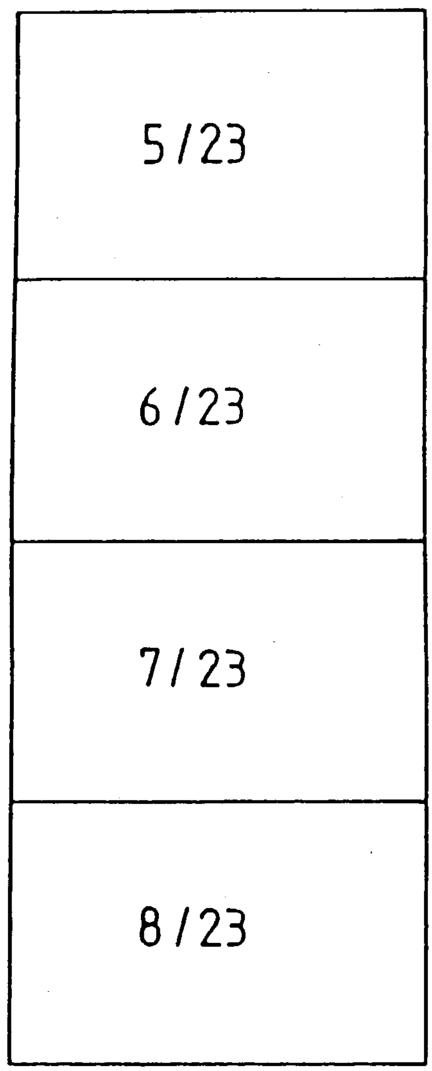

FIG. 2

1	CTCAGCGCCC	AGCCGCTTCC	TGCCTGGATT	CCACAGCTTC	GCGCCGTGTA
51	CTGTCGCCCC	ATCCCTGCGC	GCCCAGCCTG	CCAAGCAGCG	TGCCCCGGTT
101	GCAGGCGTCA	TGCAGCGGGC	GCGACCCACG	CTCTGGGCCG	CTGCGCTGAC
151	TCTGCTGGTG	CTGCTCCGCG	GGCCGCCGGT	GGCGCGGGCT	GGCGCGAGCT
201	CGGGGGGCTT	GGGTCCCGTG	GTGCGCTGCG	AGCCGTGCGA	CGCGCGTGCA
251	CTGGCCCAGT	GCGCGCCTCC	GCCCGCCGTG	TGCGCGGAGC	TGGTGCGCGA
301	GCCGGGCTGC	GGCTGCTGCC	TGACGTGCGC	ACTGAGCGAG	GGCCAGCCGT
351	GCGGCATCTA	CACCGAGCGC	TGTGGCTCCG	GCCTTCGCTG	CCAGCCGTCG
401	CCCGACGAGG	CGCGACCGCT	GCAGGCGCTG	CTGGACGGCC	GCGGGCTCTG
451	CGTCAACGCT	AGTGCCGTCA	GCCGCCTGCG	CGCCTACCTG	CTGCCAGCGC
501	CGCCAGCTCC	AGGAAATGCT	AGTGAGTCGG	AGGAAGACCG	CAGCGCCGGC
551	AGTGTGGAGA	GCCCGTCCGT	CTCCAGCACG	CACCGGGTGT	CTGATCCCAA
601	GTTCCACCCC	CTCCATTCAA	AGATAATCAT	CATCAAGAAA	GGGCATGCTA
651	AAGACAGCCA	GCGCTACAAA	GTTGACTACG	AGTCTCAGAG	CACAGATACC
701	CAGAACTTCT	CCTCCGAGTC	CAAGCGGGAG	ACAGAATATG	GTCCCTGCCG

FIG.2A

751	TAGAGAAATG	GAAGACACAC	TGAATCACCT	GAAGTTCCTC	AATGTGCTGA
801	GTCCCAGGGG	TGTACACATT	CCCAACTGTG	ACAAGAAGGG	ATTTTATAAG
851	AAAAAGCAGT	GTCGCCCTTC	CAAAGGCAGG	AAGCGGGGCT	TCTGCTGGTG
901	TGTGGATAAG	TATGGGCAGC	CTCTCCCAGG	CTACACCACC	AAGGGGAAGG
951	AGGACGTGCA	CTGCTACAGC	ATGCAGAGCA	AGTAGACGCC	TGCCGCAAGT
1001	TAATGTGGAG	CTCAAATATG	CCTTATTTTG	CACAAAAGAC	TGCCAAGGAC
1051	ATGACCAGCA	GCTGGCTACA	GCCTCGATTT	ATATTTCTGT	TTGTGGTGAA
1101	CTGATTTTTT	TTAAACCAAA	GTTTAGAAAG	AGGTTTTTGA	AATGCCTATG
1151	GTTTCTTTGA	ATGGTAAACT	TGAGCATCTT	TTCACTTTCC	AGTAGTCAGC
1201	AAAGAGCAGT	TTGAATTTTC	TTGTCGCTTC	CTATCAAAAT	ATTCAGAGAC
1251	TCGAGCACAG	CACCCAGACT	TCATGCGCCC	GTGGAATGCT	CACCACATGT
1301	TGGTCGAAGC	GGCCGACCAC	TGACTTTGTG	ACTTAGGCGG	CTGTGTTGCC
1351	TATGTAGAGA	ACACGCTTCA	CCCCCACTCC	CCGTACAGTG	CGCACAGGCT
1401	TTATCGAGAA	TAGGAAAACC	TTTAAACCCC	GGTCATCCGG	ACATCCCAAC
1451	GCATGCTCCT	GGAGCTCACA	GCCTTCTGTG	GTGTCATTTC	TGAAACAAGG

FIG.2B

1501 GCGTGGATCC CTCAACCAAG AAGAATGTTT ATGTCTTCAA GTGACCTGTA
1551 CTGCTTGGGG ACTATTGGAG AAAATAAGGT GGAGTCCTAC TTGTTTAAAA
1601 AATATGTATC TAAGAATGTT CTAGGGCACT CTGGGAACCT ATAAAGGCAG
1651 GTATTTCGGG CCCTCCTCTT CAGGAATCTT CCTGAAGACA TGGCCCAGTC
1701 GAAGGCCCAG GATGGCTTTT GCTGCGGCCC CGTGGGGTAG GAGGGACAGA
1751 GAGACGGGAG AGTCAGCCTC CACATTCAGA GGCATCACAA GTAATGGCAC
1801 AATTCTTCGG ATGACTGCAG AAAATAGTGT TTTGTAGTTC AACAACTCAA
1851 GACGAAGCTT ATTTCTGAGG ATAAGCTCTT TAAAGGCAAA GCTTTATTTT
1901 CATCTCTCAT CTTTTGTCCT CCTTAGCACA ATGTAAAAAA GAATAGTAAT
1951 ATCAGAACAG GAAGGAGGAA TGGCTTGCTG GGGAGCCCAT CCAGGACACT
2001 GGGAGCACAT AGAGATTCAC CCATGTTTGT TGAACTTAGA GTCATTCTCA
2051 TGCTTTTCTT TATAATTCAC ACATATATGC AGAGAAGATA TGTTCTTGTT
2101 AACATTGTAT ACAACATAGC CCCAAATATA GTAAGATCTA TACTAGATAA
2151 TCCTAGATGA AATGTTAGAG ATGCTATATG ATACAACTGT GGCCATGACT
2201 GAGGAAAGGA GCTCACGCCC AGAGACTGGG CTGCTCTCCC GGAGGCCAAA

FIG.2C

2251 CCCAAGAAGG TCTGGCAAAG TCAGGCTCAG GGAGACTCTG CCCTGCTGCA
2301 GACCTCGGTG TGGACACACG CTGCATAGAG CTCTCCTTGA AAACAGAGGG
2351 GTCTCAAGAC ATTCTGCCTA CCTATTAGCT TTTCTTTATT TTTTTAACTT
2401 TTTGGGGGGA AAAGTATTTT TGAGAAGTTT GTCTTGCAAT GTATTTATAA
2451 ATAGTAAATA AAGTTTTTAC CATT

FIG. 2D

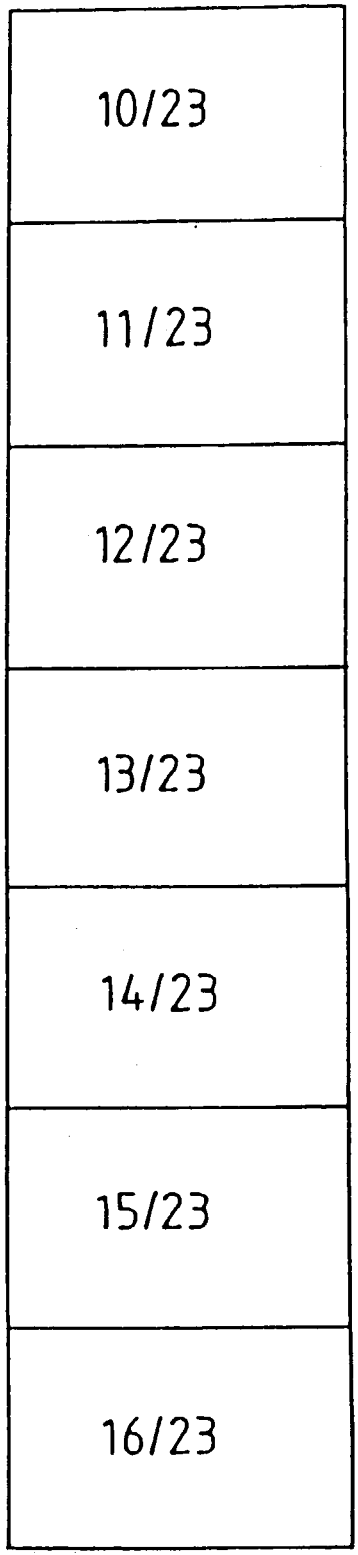

FIG. 3

1	TTTTTTTTTT	TTTTGAGAAA	GGGAATTTCA	TCCCAAATAA	AAGGAATGAA
51	GTCTGGCTCC	GGAGGAGGGT	CCCCGACCTC	GCTGTGGGGG	CTCCTGTTTC
101	TCTCCGCCGC	GCTCTCGCTC	TGGCCGACGA	GTGGAGAAAT	CTGCGGGCCA
151	GGCATCGACA	TCCGCAACGA	CTATCAGCAG	CTGAAGCGCC	TGGAGAACTG
201	CACGGTGATC	GAGGGCTACC	TCCACATCCT	GCTCATCTCC	AAGGCCGAGG
251	ACTACCGCAG	CTACCGCTTC	CCCAAGCTCA	CGGTCATTAC	CGAGTACTTG
301	CTGCTGTTCC	GAGTGGCTGG	CCTCGAGAGC	CTCGGAGACC	TCTTCCCCAA
351	CCTCACGGTC	ATCCGCGGCT	GGAAACTCTT	CTACAACTAC	GCCCTGGTCA
401	TCTTCGAGAT	GACCAATCTC	AAGGATATTG	GGCTTTACAA	CCTGAGGAAC
451	ATTACTCGGG	GGGCCATCAG	GATTGAGAAA	AATGCTGACC	TCTGTTACCT
501	CTCCACTGTG	GACTGGTCCC	TGATCCTGGA	TGCGGTGTCC	AATAACTACA
551	TTGTGGGGAA	TAAGCCCCCA	AAGGAATGTG	GGGACCTGTG	TCCAGGGACC
601	ATGGAGGAGA	AGCCGATGTG	TGAGAAGACC	ACCATCAACA	ATGAGTACAA
651	CTACCGCTGC	TGGACCACAA	ACCGCTGCCA	GAAAATGTGC	CCAAGCACGT
701	GTGGGAAGCG	GGCGTGCACC	GAGAACAATG	AGTGCTGCCA	CCCCGAGTGC

FIG. 3A

```
751   CTGGGCAGCT GCAGCGCGCC TGACAACGAC ACGGCCTGTG TAGCTTGCCG
801   CCACTACTAC TATGCCGGTG TCTGTGTGCC TGCCTGCCCG CCCAACACCT
851   ACAGGTTTGA GGGCTGGCGC TGTGTGGACC GTGACTTCTG CGCCAACATC
901   CTCAGCGCCG AGAGCAGCGA CTCCGAGGGG TTTGTGATCC ACGACGGCGA
951   GTGCATGCAG GAGTGCCCCT CGGGCTTCAT CCGCAACGGC AGCCAGAGCA
1001  TGTACTGCAT CCCTTGTGAA GGTCCTTGCC CGAAGGTCTG TGAGGAAGAA
1051  AAGAAAACAA AGACCATTGA TTCTGTTACT TCTGCTCAGA TGCTCCAAGG
1101  ATGCACCATC TTCAAGGGCA ATTTGCTCAT TAACATCCGA CGGGGGAATA
1151  ACATTGCTTC AGAGCTGGAG AACTTCATGG GGCTCATCGA GGTGGTGACG
1201  GGCTACGTGA AGATCCGCCA TTCTCATGCC TTGGTCTCCT TGTCCTTCCT
1251  AAAAAACCTT CGCCTCATCC TAGGAGAGGA GCAGCTAGAA GGGAATTACT
1301  CCTTCTACGT CCTCGACAAC CAGAACTTGC AGCAACTGTG GGACTGGGAC
1351  CACCGCAACC TGACCATCAA AGCAGGGAAA ATGTACTTTG CTTTCAATCC
1401  CAAATTATGT GTTTCCGAAA TTTACCGCAT GGAGGAAGTG ACGGGGACTA
1451  AAGGGCGCCA AAGCAAAGGG GACATAAACA CCAGGAACAA CGGGGAGAGA
```

FIG. 3B

1501	GCCTCCTGTG	AAAGTGACGT	CCTGCATTTC	ACCTCCACCA	CCACGTCGAA
1551	GAATCGCATC	ATCATAACCT	GGCACCGGTA	CCGGCCCCCT	GACTACAGGG
1601	ATCTCATCAG	CTTCACCGTT	TACTACAAGG	AAGCACCCTT	TAAGAATGTC
1651	ACAGAGTATG	ATGGGCAGGA	TGCCTGCGGC	TCCAACAGCT	GGAACATGGT
1701	GGACGTGGAC	CTCCCGCCCA	ACAAGGACGT	GGAGCCCGGC	ATCTTACTAC
1751	ATGGGCTGAA	GCCCTGGACT	CAGTACGCCG	TTTACGTCAA	GGCTGTGACC
1801	CTCACCATGG	TGGAGAACGA	CCATATCCGT	GGGGCCAAGA	GTGAGATCTT
1851	GTACATTCGC	ACCAATGCTT	CAGTTCCTTC	CATTCCCTTG	GACGTTCTTT
1901	CAGCATCGAA	CTCCTCTTCT	CAGTTAATCG	TGAAGTGGAA	CCCTCCCTCT
1951	CTGCCCAACG	GCAACCTGAG	TTACTACATT	GTGCGCTGGC	AGCGGCAGCC
2001	TCAGGACGGC	TACCTTTACC	GGCACAATTA	CTGCTCCAAA	GACAAAATCC
2051	CCATCAGGAA	GTATGCCGAC	GGCACCATCG	ACATTGAGGA	GGTCACAGAG
2101	AACCCCAAGA	CTGAGGTGTG	TGGTGGGGAG	AAAGGGCCTT	GCTGCGCCTG
2151	CCCCAAAACT	GAAGCCGAGA	AGCAGGCCGA	GAAGGAGGAG	GCTGAATACC
2201	GCAAAGTCTT	TGAGAATTTC	CTGCACAACT	CCATCTTCGT	GCCCAGACCT

FIG. 3C

2251 GAAAGGAAGC GGAGAGATGT CATGCAAGTG GCCAACACCA CCATGTCCAG
2301 CCGAAGCAGG AACACCACGG CCGCAGACAC CTACAACATC ACCGACCCGG
2351 AAGAGCTGGA GACAGAGTAC CCTTTCTTTG AGAGCAGAGT GGATAACAAG
2401 GAGAGAACTG TCATTTCTAA CCTTCGGCCT TTCACATTGT ACCGCATCGA
2451 TATCCACAGC TGCAACCACG AGGCTGAGAA GCTGGGCTGC AGCGCCTCCA
2501 ACTTCGTCTT TGCAAGGACT ATGCCCGCAG AAGGAGCAGA TGACATTCCT
2551 GGGCCAGTGA CCTGGGAGCC AAGGCCTGAA AACTCCATCT TTTTAAAGTG
2601 GCCGGAACCT GAGAATCCCA ATGGATTGAT TCTAATGTAT GAAATAAAAT
2651 ACGGATCACA AGTTGAGGAT CAGCGAGAAT GTGTGTCCAG ACAGGAATAC
2701 AGGAAGTATG GAGGGGCCAA GCTAAACCGG CTAAACCCGG GGAACTACAC
2751 AGCCCGGATT CAGGCCACAT CTCTCTCTGG GAATGGGTCG TGGACAGATC
2801 CTGTGTTCTT CTATGTCCAG GCCAAAACAG GATATGAAAA CTTCATCCAT
2851 CTGATCATCG CTCTGCCCGT CGCTGTCCTG TTGATCGTGG GAGGGTTGGT
2901 GATTATGCTG TACGTCTTCC ATAGAAAGAG AAATAACAGC AGGCTGGGGA
2951 ATGGAGTGCT GTATGCCTCT GTGAACCCGG AGTACTTCAG CGCTGCTGAT

FIG.3D

3001	GTGTACGTTC	CTGATGAGTG	GGAGGTGGCT	CGGGAGAAGA	TCACCATGAG
3051	CCGGGAACTT	GGGCAGGGGT	CGTTTGGGAT	GGTCTATGAA	GGAGTTGCCA
3101	AGGGTGTGGT	GAAAGATGAA	CCTGAAACCA	GAGTGGCCAT	TAAAACAGTG
3151	AACGAGGCCG	CAAGCATGCG	TGAGAGGATT	GAGTTTCTCA	ACGAAGCTTC
3201	TGTGATGAAG	GAGTTCAATT	GTCACCATGT	GGTGCGATTG	CTGGGTGTGG
3251	TGTCCCAAGG	CCAGCCAACA	CTGGTCATCA	TGGAACTGAT	GACACGGGGC
3301	GATCTCAAAA	GTTATCTCCG	GTCTCTGAGG	CCAGAAATGG	AGAATAATCC
3351	AGTCCTAGCA	CCTCCAAGCC	TGAGCAAGAT	GATTCAGATG	GCCGGAGAGA
3401	TTGCAGACGG	CATGGCATAC	CTCAACGCCA	ATAAGTTCGT	CCACAGAGAC
3451	CTTGCTGCCC	GGAATTGCAT	GGTAGCCGAA	GATTTCACAG	TCAAAATCGG
3501	AGATTTTGGT	ATGACGCGAG	ATATCTATGA	GACAGACTAT	TACCGGAAAG
3551	GAGGCAAAGG	GCTGCTGCCC	GTGCGCTGGA	TGTCTCCTGA	GTCCCTCAAG
3601	GATGGAGTCT	TCACCACTTA	CTCGGACGTC	TGGTCCTTCG	GGGTCGTCCT
3651	CTGGGAGATC	GCCACACTGG	CCGAGCAGCC	CTACCAGGGC	TTGTCCAACG
3701	AGCAAGTCCT	TCGCTTCGTC	ATGGAGGGCG	GCCTTCTGGA	CAAGCCAGAC

FIG.3E

```
3751  AACTGTCCTG ACATGCTGTT TGAACTGATG CGCATGTGCT GGCAGTATAA
3801  CCCCAAGATG AGGCCTTCCT TCCTGGAGAT CATCAGCAGC ATCAAAGAGG
3851  AGATGGAGCC TGGCTTCCGG GAGGTCTCCT TCTACTACAG CGAGGAGAAC
3901  AAGCTGCCCG AGCCGGAGGA GCTGGACCTG GAGCCAGAGA ACATGGAGAG
3951  CGTCCCCCTG GACCCCTCGG CCTCCTCGTC CTCCCTGCCA CTGCCCGACA
4001  GACACTCAGG ACACAAGGCC GAGAACGGCC CCGGCCCTGG GGTGCTGGTC
4051  CTCCGCGCCA GCTTCGACGA GAGACAGCCT TACGCCCACA TGAACGGGGG
4101  CCGCAAGAAC GAGCGGGCCT TGCCGCTGCC CCAGTCTTCG ACCTGCTGAT
4151  CCTTGGATCC TGAATCTGTG CAAACAGTAA CGTGTGCGCA CGCGCAGCGG
4201  GGTGGGGGGG GAGAGAGAGT TTTAACAATC CATTCACAAG CCTCCTGTAC
4251  CTCAGTGGAT CTTCAGTTCT GCCCTTGCTG CCCGCGGGAG ACAGCTTCTC
4301  TGCAGTAAAA CACATTTGGG ATGTTCCTTT TTTCAATATG CAAGCAGCTT
4351  TTTATTCCCT GCCCAAACCC TTAACTGACA TGGGCCTTTA AGAACCTTAA
4401  TGACAACACT TAATAGCAAC AGAGCACTTG AGAACCAGTC TCCTCACTCT
4451  GTCCCTGTCC TTCCCTGTTC TCCCTTTCTC TCTCCTCTCT GCTTCATAAC
```

FIG.3F

```
4501  GGAAAAATAA TTGCCACAAG TCCAGCTGGG AAGCCCTTTT TATCAGTTTG
4551  AGGAAGTGGC TGTCCCTGTG GCCCCATCCA ACCACTGTAC ACACCCGCCT
4601  GACACCGTGG GTCATTACAA AAAAACACGT GGAGATGGAA ATTTTTACCT
4651  TTATCTTTCA CCTTTCTAGG GACATGAAAT TTACAAAGGG CCATCGTTCA
4701  TCCAAGGCTG TTACCATTTT AACGCTGCCT AATTTTGCCA AAATCCTGAA
4751  CTTTCTCCCT CATCGGCCCG GCGCTGATTC CTCGTGTCCG GAGGCATGGG
4801  TGAGCATGGC AGCTGGTTGC TCCATTTGAG AGACACGCTG GCGACACACT
4851  CCGTCCATCC GACTGCCCCT GCTGTGCTGC TCAAGGCCAC AGGCACACAG
4901  GTCTCATTGC TTCTGACTAG ATTATTATTT GGGGGAACTG GACACAATAG
4951  GTCTTTCTCT CAGTGAAGGT GGGGAGAAGC TGAACCGGC
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FIG.3G

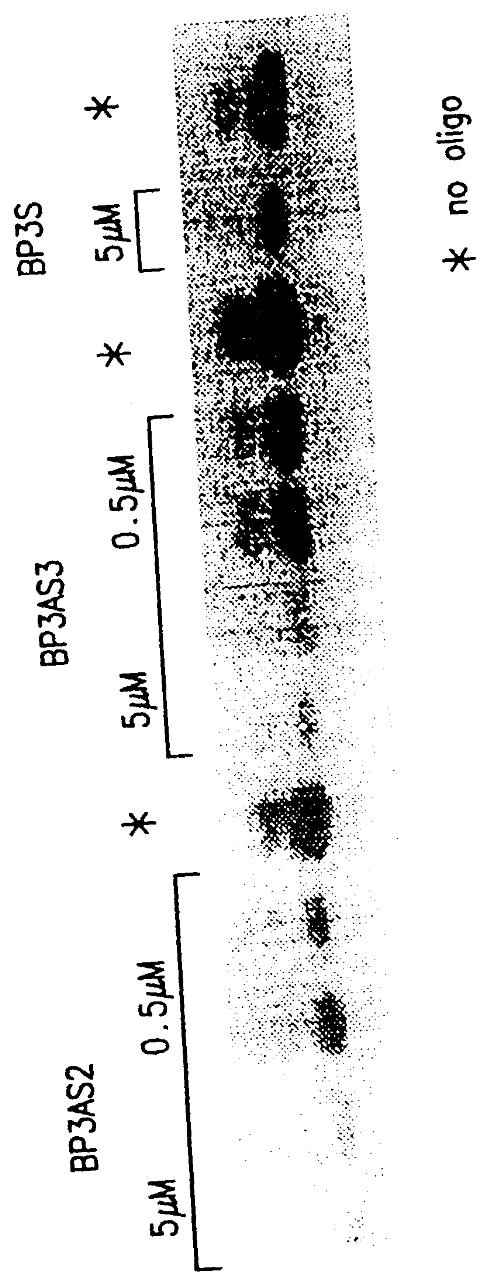

FIG.4A

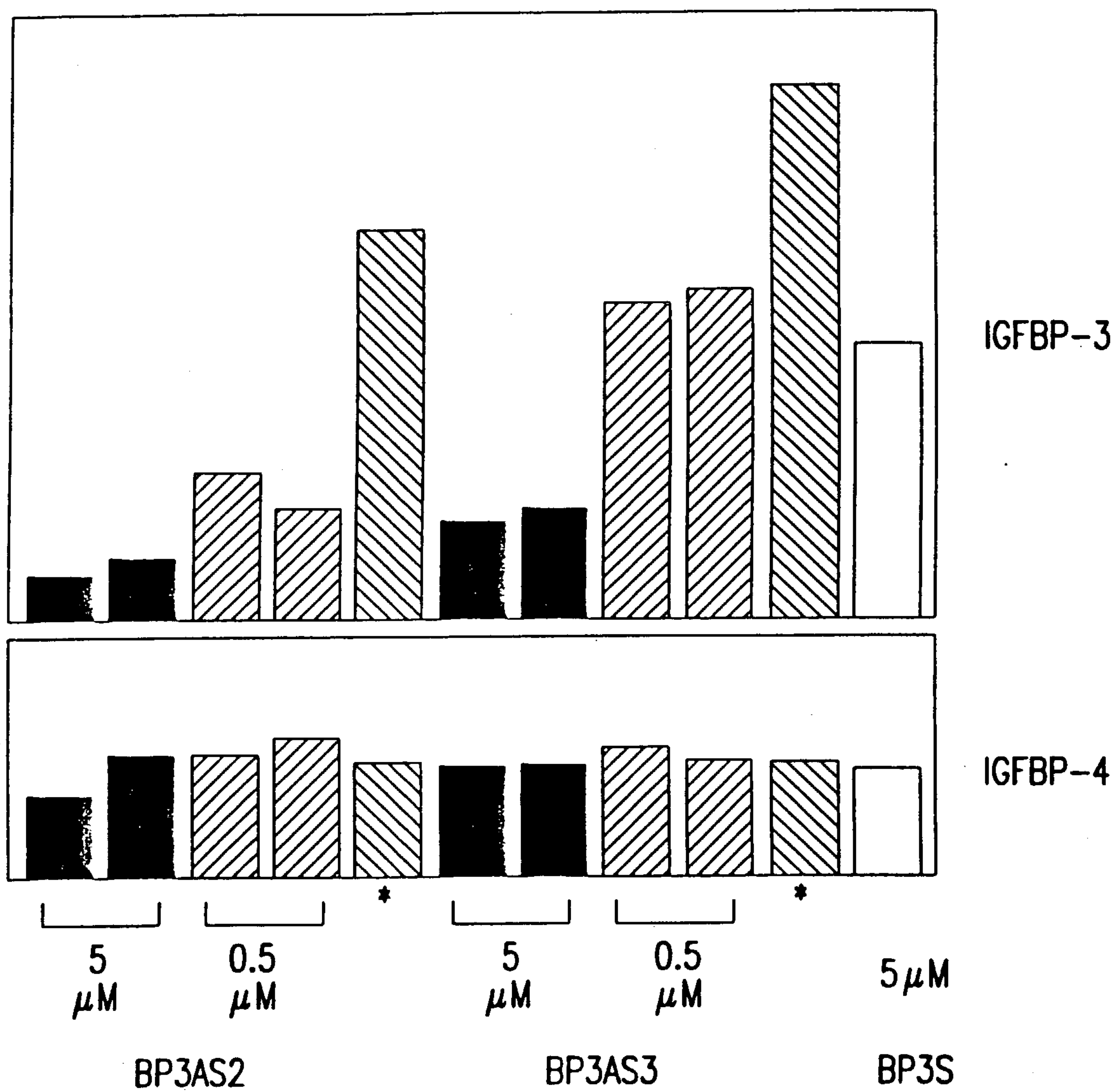

FIG.4B

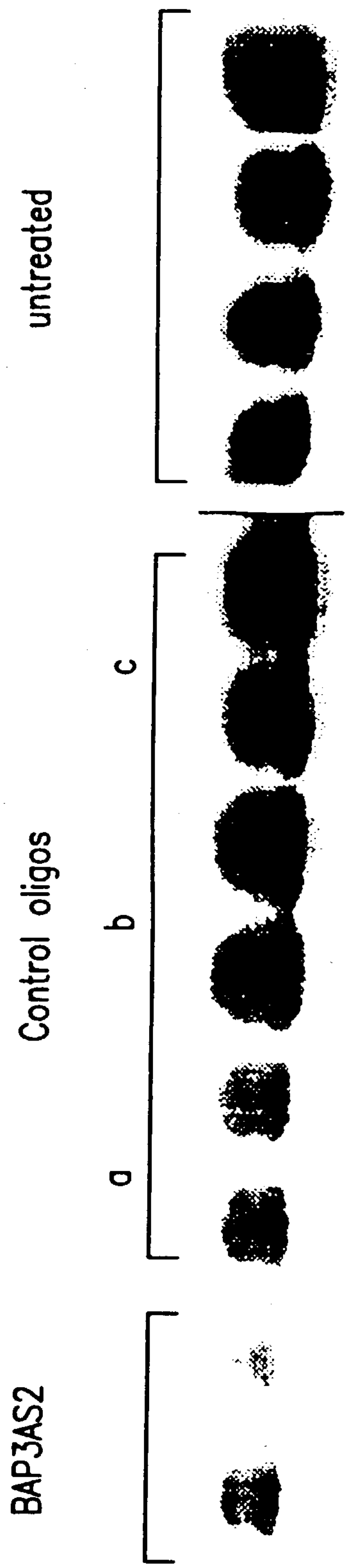

FIG.5A

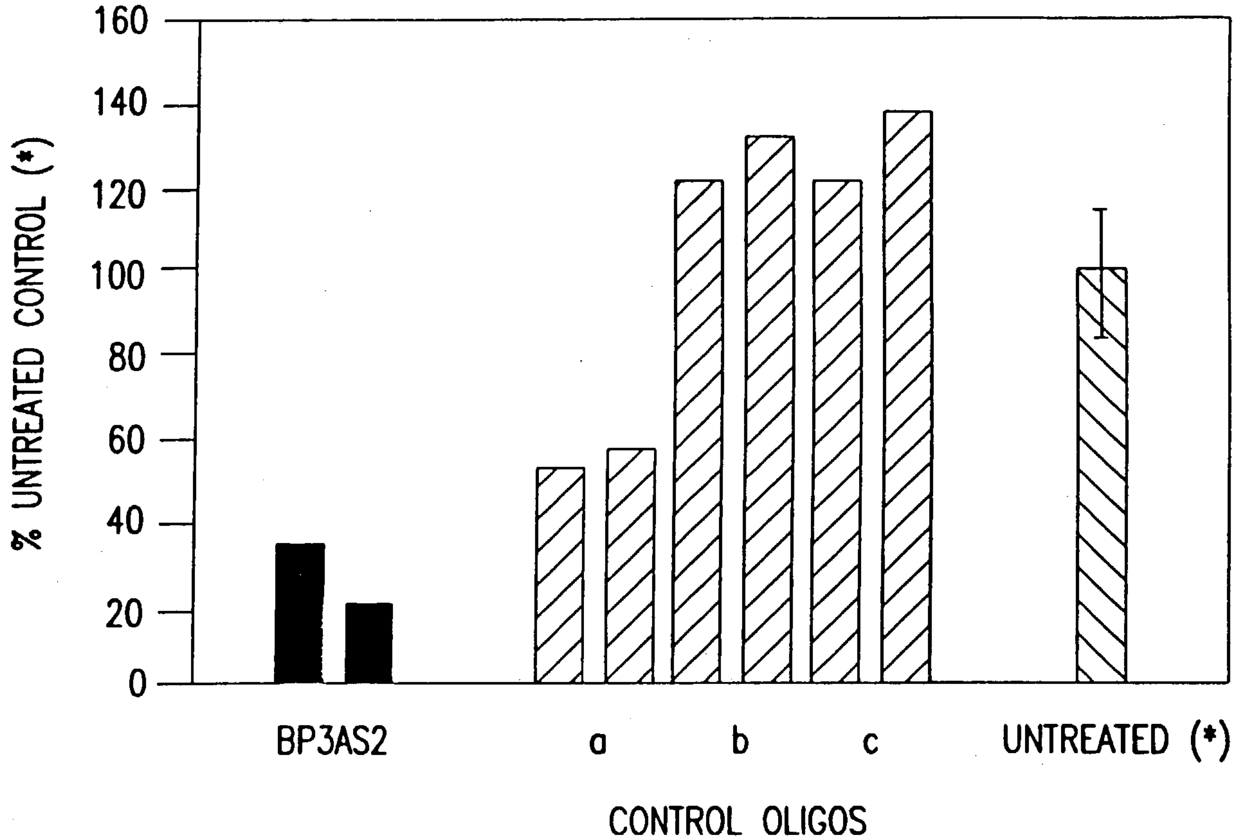
160
140
120
100
80
60
40
20
0
% UNTREATED CONTROL (*)
BP3AS2
a
b
c
UNTREATED (*)
CONTROL OLIGOS

FIG.5B

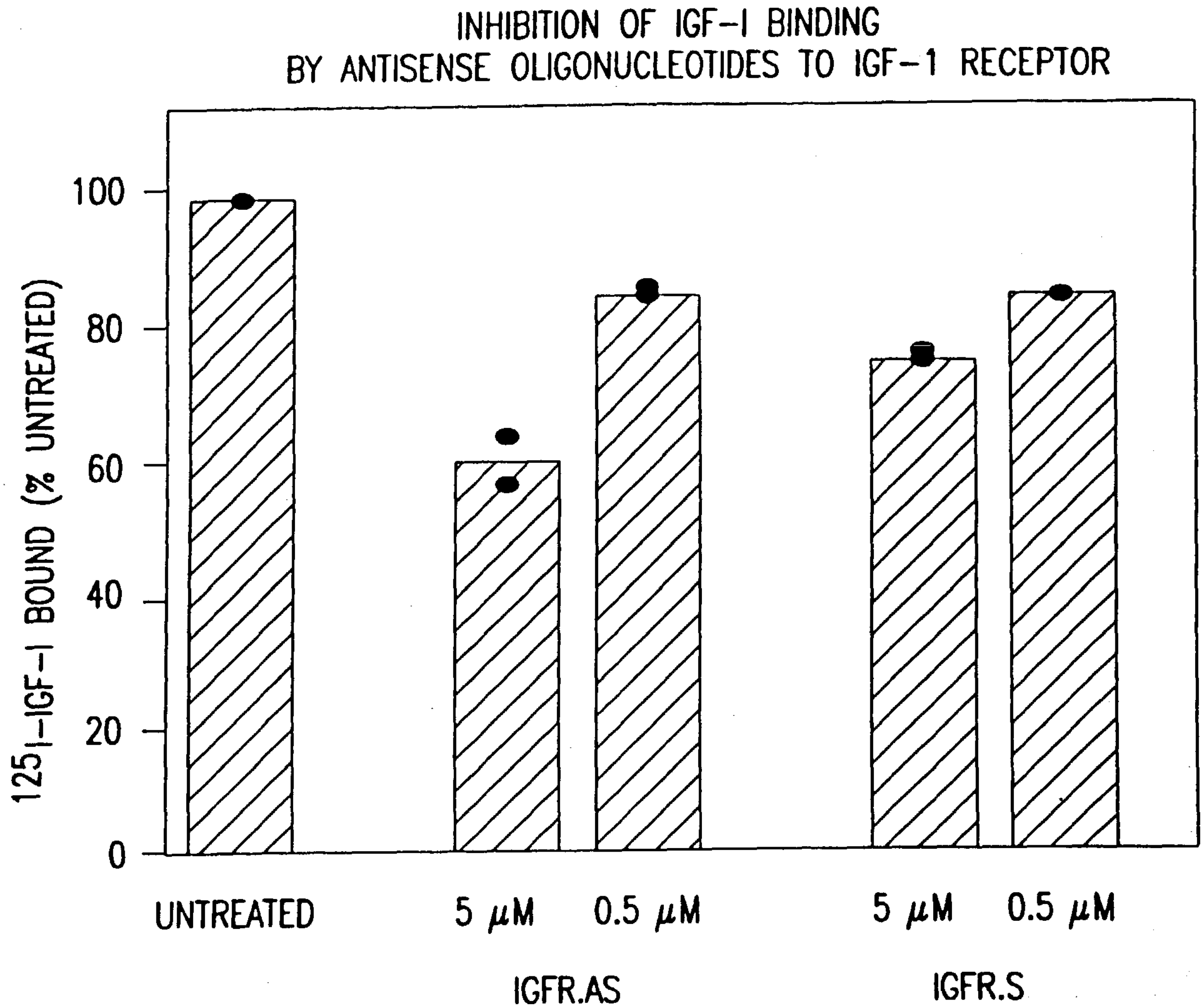
INHIBITION OF IGF-I BINDING
BY ANTISENSE OLIGONUCLEOTIDES TO IGF-1 RECEPTOR
125I-IGF-I BOUND (% UNTREATED)
100
80
60
40
20
0
UNTREATED
5 μM
0.5 μM
5 μM
0.5 μM
IGFR.AS
IGFR.S

FIG.6

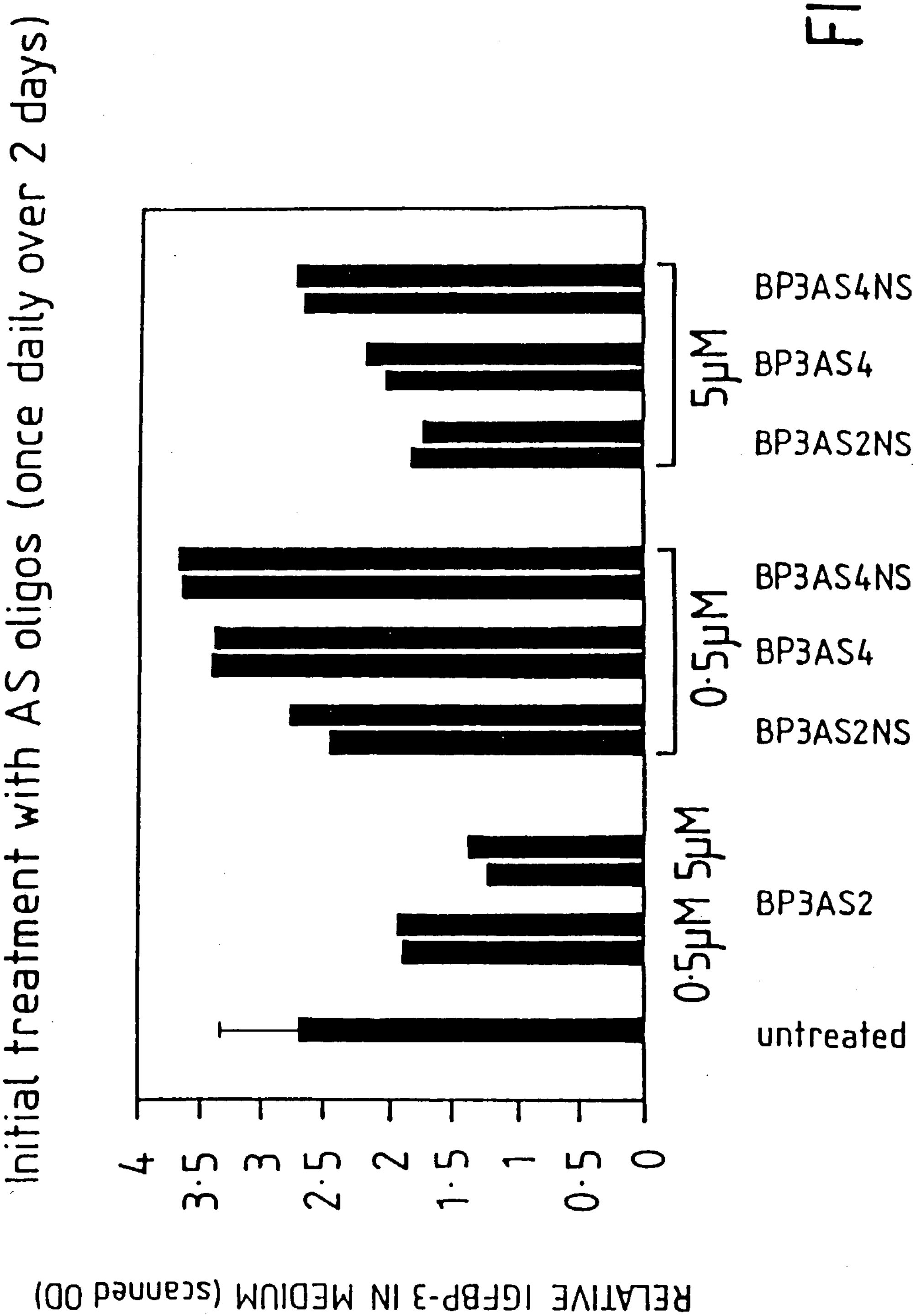
Initial treatment with AS oligos (once daily over 2 days)
RELATIVE IGFBP-3 IN MEDIUM (scanned OD)
0
0·5
1
1·5
2
2·5
3
3·5
4
untreated
0·5μM 5μM
BP3AS2
0·5μM
BP3AS2NS
BP3AS4
BP3AS4NS
5μM
BP3AS2NS
BP3AS4
BP3AS4NS

FIG.7

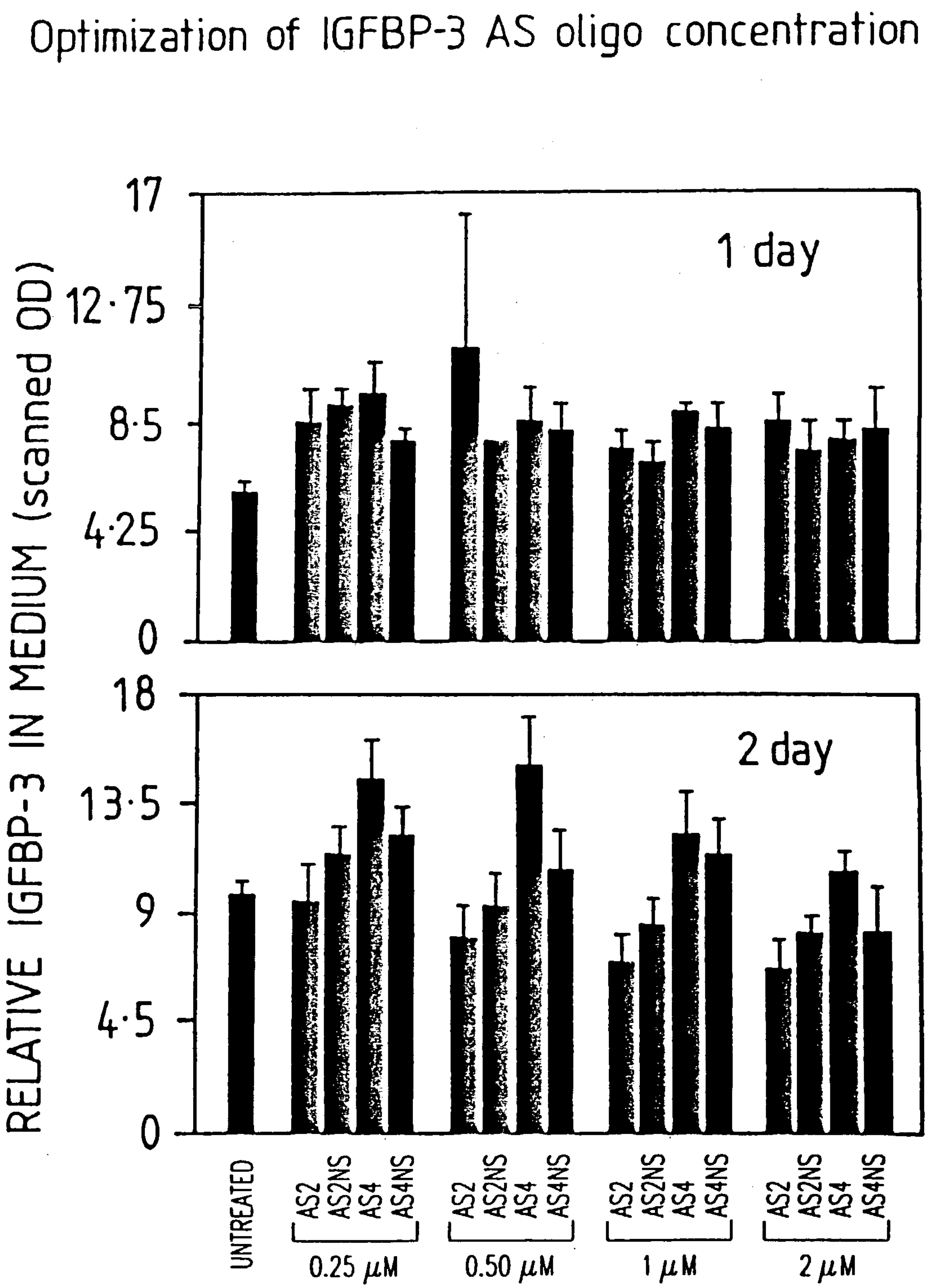
Optimization of IGFBP-3 AS oligo concentration
RELATIVE IGFBP-3 IN MEDIUM (scanned OD)
1 day
2 day
17
12·75
8·5
4·25
0
18
13·5
9
4·5
0
UNTREATED
AS2
AS2NS
AS4
AS4NS
0.25 μM
0.50 μM
1 μM
2 μM

FIG. 8